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Green Sample Preparation in Analytical Separation Sciences: Electrophoretic Concentration

by

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Abstract

Traditional sample preparation requires substantial resources and time, both adversely affecting the economical and ecological accounts of an analytical workflow. To address the dearth of greenness, this work used field-enhanced and electrokinetic sample injection from capillary electrophoresis (CE) for off-line sample preparation. This approach, referred to as electrophoretic concentration (EC) and simultaneous EC and separation (SECS), relies on the use of an electric field to transfer charged analytes from a mL-volume of aqueous sample to 20 µL of acceptor electrolyte immobilised in a micropipette. The use of a conductive hydrogel to facilitate a zero net-flow inside a fused silica capillary is described and then explored for EC of charged analytes. The hydrogel was crucial to the success of EC, because it supported voltage application and retained the acceptor electrolyte in the micropipette. Anionic dyes and pollutants from drinking water as well as cationic drugs from wastewater were concentrated in less than 50 min and sensitive analysis by CE was achieved. The EC setup was then modified for SECS and implemented on an eight channel device to increase the sample throughput. Herbicides fortified in river water and beer samples were used to study SECS in combination with chromatographic and electrophoretic separation employing UV and mass spectrometric detection. Analyte enrichments of up to a factor of 337 in less than 45 min were achieved which enabled low ng/mL detection. Compared to solid-phase extraction, SECS reduced the sample preparation time by 94% and resource consumption by 99%. EC and SECS in combination with stacking-CE showed potential for trace analysis and all the SECS and EC acceptor electrolytes were directly compatible for analytical separation without the need for time-consuming steps. EC and SECS were organic solvent-free, rapid and simple sample preparations which were complying with the principles of Green Analytical Chemistry.

Declaration of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Candidate was the primary author and Author 1 and Author 2 contributed to the idea, its formalisation and revision.

Paper 2: A. Wuethrich, P.R. Haddad, J.P. Quirino, Zero net-flow in capillary electrophoresis using acrylamide based hydrogel, *Analyst*. 139 (2014), 3722–3726.

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Located in Chapter 6

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Content

List of abbreviations

26N	2,6-naphthalenedisulfonic acid disodium salt
2N	3-hydroxynaphthalene-2,7-disulfonic acid
3PLE	three-phase liquid electroextraction
7N	1,3,(6,7)-naphthalenetrisulfonic acid trisodium salt
AEM	anion-exchange membrane
AMPA	aminomethylphosphonic acid
ASM	anion-selective membrane
BGE	background electrolyte
CE	capillary electrophoresis
CEM	cation-exchange membrane
CF	concentration factor
cITP	capillary isotachophoresis
CSM	cation-selective membrane
CZE	capillary zone electrophoresis
DEP	dielectrophoresis
Dichlorprop	2-(2,4-dichlorophenoxy)propionic acid
DIN	dissolved inorganic nitrogen
DLLME	dispersive liquid-liquid microextraction
DNA	deoxyribonucleic acid
DON	dissolved organic nitrogen
EC	electrophoretic concentration
ED	electrodialysis
EF	electrofiltration
EFA	electric field-assisted

ELC	electrochemical
EME	electromembrane extraction
EMF	electromicrofiltration
EOF	electroosmotic flow
EU	European Union
Fenoprop	2-(2,4,5-trichlorophenoxy)propionic acid
FESI	field-enhanced/amplified sample injection
FLM	free liquid membrane
GC	gas chromatography
HF	hollow fibre
HPLC	high performance liquid chromatography
HV	high voltage
ICP	ion concentration polarisation
K	coverage factor
LC	liquid chromatography
LLE	liquid-liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
LPME	liquid-phase microextraction
MAE	microwave-assisted extraction
MDL	method detection limit
Mecoprop	2-(4-chloro-2-methylphenoxy)propionic acid
MEKC	micellar electrokinetic chromatography
MQL	method quantitation limit
MS	mass spectrometry
MSS	micelle to solvent stacking
MW	molecular weight

n	number of measurements
NSM	non-selective membrane
PDMS	polydimethylsiloxane
PLE	pressurised liquid extraction
R^2	coefficient of determination
RSD	relative standard deviation
S/N	signal to noise ratio
SBSE	stir bar sorptive extraction
SD	standard deviation
SDME	single-drop microextraction
SDS	sodium dodecyl sulfate
SECS	simultaneous electrophoretic concentration and separation
SEF	sensitivity enhancement factor
SI	supporting Information
SLE	solid-liquid extraction
SLM	supported liquid membrane
SPE	solid-phase extraction
SPME	solid-phase microextraction
TN	total nitrogen
Tris	2-amino-2-hydroxymethyl-propane-1,3-diosodium hydroxide
U	uncertainty associated with repeatability
UAE	ultrasound-assisted extraction
USP	United States Pharmacopeia
UV	ultraviolet
V	4-vinylbenzenesulfonic acid

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Peer-reviewed articles

1. A. Wuethrich, P.R. Haddad, J.P. Quirino, The electric field – an emerging driver in sample preparation, *TrAC Trends Anal. Chem*. 80 (2016), 604-611. (Chapter 1)
2. A. Wuethrich, P.R. Haddad, J.P. Quirino, Zero net-flow in capillary electrophoresis using acrylamide based hydrogel, *Analyst*. 139 (2014), 3722–3726. (Chapter 2)
3. A. Wuethrich, P.R. Haddad, J.P. Quirino, Off-line sample preparation by electrophoretic concentration using a micropipette and hydrogel, *J. Chromatogr. A*. 1369 (2014), 186–190. (Chapter 3)
4. A. Wuethrich, P.R. Haddad, J.P. Quirino, Electrophoretic concentration and sweeping-micellar electrokinetic chromatography analysis of cationic drugs in water samples, *J. Chromatogr. A*. 1401 (2015), 84–88. (Chapter 4)
5. A. Wuethrich, P.R. Haddad, J.P. Quirino, Green Sample Preparation for Liquid Chromatography and Capillary Electrophoresis of Anionic and Cationic Analytes, *Anal. Chem*. 87 (2015), 4117–4123. (Chapter 5)
6. A. Wuethrich, P.R. Haddad, J.P. Quirino, Simultaneous electrophoretic concentration and separation of herbicides in beer prior to stacking-capillary electrophoresis-UV and liquid chromatography-mass spectrometry, *Electrophoresis*. 37 (2016), 1122-1128. (Chapter 6)

Oral presentation

A. Wuethrich, P.R. Haddad, J.P. Quirino, 14th Asia-Pacific International Symposium on Microscale Separations and Analysis, Kyoto, Japan, 8-10 December 2014. Title: *Off-line Sample Preparation by Electrophoretic Concentration using a Micropipette and Hydrogel*.

Poster presentations

A. Wuethrich, P.R. Haddad, J.P. Quirino, 40th International Symposium on High Performance Liquid Phase Separations and Related Techniques (HPLC 2013 Hobart), Hobart, Australia, 18-21 November 2013. Title: *Suppressed electroosmotic flow in capillary electrophoresis using hydrogel*.

Introduction

In analytical chemistry, separation science is a sub-category which delivers essential qualitative and quantitative information for the pharmaceutical, environmental, clinical and other sectors. In this sub-category, a typical workflow consists of sampling, sample preparation, analyte separation and detection, and data processing. The sample preparation step is crucial because it transforms the analyte into a suitable state for separation/detection and thus it directly influences the quality of the analytical result. This step accounts for up to 80% of the workflow time and it uses substantial resources with potential detrimental effects on the environment.[1] The introduction of Green Chemistry led to a paradigm shift in chemistry and also promoted the field of Green Analytical Chemistry, including environmentally benign sample preparation.[2–5] The aim is to establish sample preparations that reduce the consumption of resources and minimise environmental pollution.

The physical state of the sample determines the strategy for sample preparation. Sample purification and analyte concentration are two strategies which aim to remove contaminants from the sample and transfer the analyte from the sample to an acceptor phase, respectively. Sample extraction can involve both purification and/or analyte concentration. Common extraction techniques are solid-liquid extraction (i.e., Soxhlet extraction) (SLE), liquid-liquid extraction (LLE) and solid-phase extraction (SPE). In Soxhlet extraction, a solid sample is continuously extracted with a recycling condensate of hot acceptor phase (e.g., organic solvent). The analyte is readily soluble in the acceptor phase while matrix components are less soluble. In LLE, an extraction cycle is performed by vigorously mixing the liquid sample with an immiscible acceptor phase prior to phase separation. This cycle can be repeated in order to increase the analyte extraction. The acceptor phase(s) is/are then combined and transferred for analysis or drying, as appropriate. In SPE, the sample is brought into contact with a solid acceptor phase which retains the target analytes. Undesired and weakly retained compounds

are washed away prior to elution of the target analytes, evaporation of the elution solvent and reconstitution of the dried analytes with a suitable solution for analysis.

The majority of environmentally-friendly sample preparation approaches have been based on modifications of SLE, LLE or SPE with the focus on reducing the solvent consumption and increasing the extraction efficiency.[6] These aims have been achieved by a change of the physical extraction conditions or by miniaturisation of the sample preparation. For the former, heat, pressure, ultrasound and microwave irradiation have been applied and these techniques were termed as pressurised liquid extraction (PLE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE). In PLE, heat and pressure are used to improve the extraction of a solid sample with a liquid acceptor phase.[7] The sample is housed in a closed cell and heated to temperatures up to 200 °C and pressurized up to 200 bars. The pressure and temperature are kept below the critical point of the acceptor phase. PLE can be applied to organic compounds of moderate to low volatility including carbohydrates, phenolic compounds, drugs of abuse, and pollutants from various samples (i.e., vegetables, plant material, soil, and air particulates).[8–11] The beneficial properties of PLE compared to Soxhlet extraction have been demonstrated for the analysis of polycyclic aromatic hydrocarbons from polyurethane.[12] The PLE approach was compared to a standard Soxhlet method (i.e., TO-13A, US Environmental Protection Agency) and resulted in a 70 and 12-times faster extraction (15 min instead of 18 h) and less consumption of acceptor phase (30 mL instead of 350 mL), respectively.

In UAE the liquid or solid sample is extracted with a liquid acceptor phase supported by ultrasonication.[13] The frequency and power of irradiation causes cavitation and micro-streaming of the acceptor phase which enhances the analyte extraction. Several classes of organic and inorganic analytes can be isolated from many different matrices.[14–16] An organic solvent-free UAE approach has been reported for quinolones and fluoroquinolone antibiotics from soil samples.[17] This approach used an aqueous solution of 0.5 g/g $Mg(NO_3)_2$ in 4% of ammonia for analyte extraction. In MAE, a solid or liquid sample is extracted with a polar

solvent or mixture of polar and apolar solvents as acceptor phase.[18,19] The acceptor phase absorbs the microwaves and is thus heated up rapidly. MAE can be conducted in an open or closed sample vessel configuration whereby the latter offers extraction at temperatures and pressures above the boiling point of the acceptor phase and atmospheric pressure, respectively. MAE has been used for analysis of heavy metals and other inorganics by sample digestion and for the extraction of organic molecules from environmental, plant and food sources.[20–23] A soft and energy-saving MAE method was reported which avoided the degradation of the polyphenol analytes obtained from *Eclipta prostrata*.[24] Comprehensive and specific reviews with the focus on environmentally-friendly PLE[25], UAE[26], and MAE [27] were published recently.

Although the approaches of PLE, UAE and MAE have demonstrated important ecological advantages, a change to harsher physical conditions has also been shown to adversely affect the analyte extraction.[6,28–32] The application of high temperatures or high energy irradiation resulted in the extraction of undesired matrix compounds (e.g., co-extraction interferences) or degradation of the analyte molecule. In addition, these approaches use instruments of medium to high capital cost and consume volumes of organic solvent in the range of 10-100 mL for each sample. In the case of UAE or MAE, an additional extract clean-up step is frequently employed to improve the extract purity. UAE is relatively slow (i.e., 60 min) compared to MAE (i.e., 10 min).[33]

For miniaturised sample preparation, the volume of acceptor phase is reduced, which increased the contact surface ratio of acceptor phase to sample and also decreased the involved consumables. Miniaturised and virtually organic solvent-free sample preparation methods include solid-phase microextraction (SPME) [34,35], stir-bar sorptive extraction (SBSE) [36], single drop microextraction (SDME) [37,38], hollow-fibre liquid-phase microextraction (HF-LPME) [39,40], and dispersive liquid-liquid extraction (DLLME) [41]. Recent reviews with emphasis on the ecological aspects of miniaturised sample preparation have been published.[42,43] In SPME and SBSE, solid absorbent materials were used as the acceptor phase which was immersed in the

liquid sample. SDME and DLLME use µL-volume of a water-immiscible liquid acceptor phase that is also submerged in or placed as a droplet in the headspace of the liquid sample. In HF-LPME, the water-immiscible acceptor phase is either in direct contact with the sample (i.e., two-phase configuration) or the aqueous acceptor phase is separated by a water-immiscible solvent from the sample (i.e., three-phase configuration).[44] In both configurations, the hollow fibre is immersed in the sample.

SPME is one of the most prevalent and mature techniques which is applied routinely in many laboratories. This technique has been used mainly for the extraction of non-polar and volatile analytes prior to gas chromatographic (GC) or liquid chromatographic (LC) separation.[45,46] After SPME, the analytes are desorbed from the fibre by thermal energy or with the use of an elution solvent. A completely organic solvent-free SPME approach has been demonstrated by thermal desorption of the analyte from the SPME fibre prior to GC analysis.[47] The SPME-fibre was modified with an ionic liquid and applied for extraction of chlorophenols from landfill leachate. The fibre could be re-used more than 80-times, which further improved the environmental-friendliness. In SBSE, a stir bar coated with the solid acceptor phase (e.g., polydimethylsiloxane) is used for non-polar to medium-polar analytes from liquid samples of all analytical fields.[48] SBSE has been shown to be efficient (i.e., high analyte enrichment) for solventless sample preparation.[49] A stir bar coated with oleic acid modified cobalt ferrite magnetic nanoparticles was used for lipophilic analytes in water samples and the approach provided analyte enrichments of up to 690. After extraction, direct thermal desorption of the acceptor phase allowed a streamlined and green workflow.

In SDME, 1-3 µL of acceptor phase are placed at the tip of a needle from a microsyringe.[44,50] In the headspace configuration, volatile and semi-volatile analytes from a wide range of liquid samples including alcoholic beverages, fragrances, essential oils, biological fluids and environmental waters have been studied.[45,51,52] In direct immersion SDME, non-polar to semi-polar compounds from various aqueous samples have been extracted.[53–55] This

approach used minimal quantities of organic solvent and improved the analyte sensitivity to relevant levels for fast and sensitive wine screening.[56] Six organophosphate insecticides were extracted from wine samples in less than 12 min by immersion of 2 µL acceptor phase (i.e., isooctane), withdrawal of the acceptor phase and direct analysis by GC-mass spectrometry (MS).

In HF-LPME, the two- and three-phase configurations are suitable for extraction of analytes of medium to high hydrophobicity from biological and environmental sources.[57–59] HF-LPME provides excellent sample clean-up and high analyte enrichment values, thus there is typically no further treatment of the acceptor phase required before analysis.[44,60] In the three-phase configuration, an anti-diabetic drug was concentrated from urine and plasma samples and analyte enrichments values of up to 280 were obtained. The acceptor phase was directly transferred for analysis by capillary electrophoresis (CE)-UV and LC-UV.[61] In DLLME, the acceptor phase and mL-volumes of a disperser solvent are introduced in the sample under strong agitation to create a dispersive solution.[62] The disperser solvent is partially miscible in both the sample and acceptor phase. Centrifugation is applied to isolate the acceptor phase prior to analysis. This approach has been used for the extraction of low polarity and hydrophobic analytes from various aqueous samples.[63] DLLME is fast and provides high analyte enrichment values. In an extraction time of only 30 s, six pyrethroid insecticides were enriched up to 84-times from fruit juices.[64]

Although these miniaturised approaches strongly support Green Analytical Chemistry, the use of minute volumes of organic solvent, delicate acceptor phase materials and the involvement of manual handling negatively affect the extraction.[44,48,65] Automation of the workflow is difficult and manual extraction procedures are common, which increases the susceptibility to errors. In SPME and SBSE, the acceptor phases were reusable, but of substantial costs of purchase. The reusability of the acceptor phase is prone to analyte carry-over which frequently requires a long pre-conditioning of the acceptor phase. The fragile

materials of the solid acceptor phase have also been noted to reduce the robustness of the method.

The selectivity of acceptor phase material restricts the sample preparation to selected groups of analytes or requires the use of different acceptor materials. In SBSE, the extraction procedure typically takes > 60 min. In SDME and HF-LPME, analyte mass transfer is relatively slow. In the case of SDME, the acceptor phase drop is small and static and thus the amount of analytes extracted is limited. Furthermore, the stability of the acceptor drop at the tip of the needle is prone to dislodging by stirring or the presence of particles in the sample. In HF-LPME, special attention has been paid during the impregnation of the HF with water-immiscible solvent and the withdrawal of the acceptor phase after extraction. The former was crucial to avoid air bubbles on the surface of the HF which decreased the analyte transfer across the HF. The latter was shown to affect the repeatability of the extraction. In DLLME, the selectivity of the approach is low which causes co-extraction of matrix compounds. Thus, the application of DLLME to complex samples has been limited or has involved the use of a second step to clean-up the extract after DLLME.

In summary, the previously mentioned approaches have in common that the main driving force for analyte extraction was the distribution coefficient between sample and acceptor phase. This is also a limitation and, as concluded by Berton and co-workers, one of the most important disadvantages faced for environmentally-friendly sample preparation is their lack of selectivity.[6] Therefore, the use of an electric field as an additional driving force could further improve analyte extraction and also introduce selectivity to the sample preparation. The electric field causes the migration of charged analytes depending on their electrophoretic mobility. A review on electric field-assisted sample preparation approaches is provided in Chapter 1. The use of an electric field for on-line sample concentration or stacking in CE is widely used, however, its potential has not yet been explored for off-line sample preparation, as accurately stated by Chen and co-workers[66]:

'Stacking is originally explored to increase the detection sensitivity of CE by increasing sample loading, but it is actually a new type of sample preparation route waiting for exploration since it can tremendously concentrate analytes into a tiny zone.'

This statement built the inspiration and motivation for this PhD thesis. Our interpretation was to develop a purely aqueous and electric field-driven scheme for off-line sample preparation of charged analytes which is referred to as *electrophoretic concentration* (EC) and *simultaneous EC and separation* (SECS). In CE, field-amplified or field-enhanced sample injection (FESI) is one form of stacking which is performed by electrokinetic injection of a low conductivity aqueous sample into a high conductivity separation electrolyte inside the CE capillary.[67] The implementation of stacking for off-line sample preparation has required addressing three main challenges, as follows: (1) the presence of an electroosmotic flow (EOF) biases the electrokinetic injection of the analytes. A strategy was developed to suppress the EOF which is described in Chapter 2; (2) the sample and acceptor phase are both aqueous and in direct contact with each other. A strategy to avoid solubilisation of the two phases was established and is presented in Chapter 3; and (3), after sample preparation the acceptor phase should be readily transferrable for analysis. This strategy is also described in Chapter 3. The chapter outline of this dissertation is as follows.

In Chapter 1, relevant literature about electric field-assisted sample preparation, including approaches for laboratory and microchip-scale, are discussed and trends are highlighted. This review has been written after the experimental Chapters 2-6 were published and thus also reviews the conducted research of this dissertation.

In Chapter 2, the use of a hydrogel to maintain a zero net-flow (i.e., sum of EOF and hydrodynamic fluid flow) inside a glass capillary is described. A zero net-flow is important because the presence of an EOF would have an adverse effect on the electrokinetic sample injection. The experiments were performed on a commercial CE instrument using fused silica capillaries and the hydrogel was prepared in the sample vial. The investigations on anionic

drugs and inorganic anions included FESI in counter-EOF capillary zone electrophoresis (CZE), the effect of polarity switching on the analytes' electrophoretic mobility, the effect of the hydrogel composition as well as the dependence of the peak shape on the pH of the separation electrolyte.

In Chapter 3, the implementation of FESI and zero net-flow conditions for off-line EC of anionic pollutants using a hydrogel and a glass micropipette is described. The hydrogel and micropipette were crucial to avoid solubilisation of the acceptor electrolyte in the sample. This chapter is a proof-of-concept study which included the study of voltage and voltage application time on the analyte concentration factor. The anionic concentrates after EC were analysed by CZE-UV. Under optimised conditions, the analytical figures of merit were determined prior to evaluation of EC on different water samples.

In Chapter 4, the concept of EC was evaluated for the sensitive analysis of five cationic drugs in purified water and wastewater. The aim of this chapter was to achieve very low method detection limits in a simple analytical workflow. The analysis of the EC concentrate was by micellar electrokinetic chromatography-UV and employed sweeping as a second sample concentration strategy. In EC, the type and concentration of the acceptor phase and the voltage application time were investigated. The method for sweeping was optimised and included the study of the injection time and the effect of acidic buffer addition to the concentrate.

In Chapter 5, the setup for EC was developed for simultaneous sample preparation of basic and acidic herbicides from river water. This chapter describes the second generation of the setup in order to allow SECS of up to eight samples in parallel. The cationic and anionic SECS-concentrates were analysed by CZE-UV and LC-UV, respectively. The SECS procedure was optimised by plotting the analyte concentration factor (y-axis) versus the investigated parameters. The studied parameters included the effect of stirring, acceptor electrolyte concentration and voltage application time. Under optimised conditions, the analytical figures of merit, intermediate precision, repeatability and uncertainty associated with repeatability

were determined. In addition, a comparison of the ecological as well as economical aspects of SECS and SPE was compiled.

In Chapter 6, the applicability of SECS as an environmentally-friendly, fast and simple sample preparation for quaternary ammonium herbicides and organophosphonate herbicides in beer was investigated. The full analytical workflow consisted of SECS followed by two-step stacking-CZE-UV and LC-MS/MS. In SECS, the concentration and pH of the acidic and basic acceptor electrolytes, and voltage application time were studied. For LC-MS/MS, an existing method was adapted with slight modification on the gradient elution. In two-step stacking, the injection length of the micellar solution, sample solution, and organic solvent phase were investigated. Under optimised conditions, the analytical performance, including accuracy values obtained by standard addition method, was determined.

In the Conclusion, Chapters 2-6 are summarised and a discussion about the potential and limitations of EC and SECS, as well as comments on their future directions, are stated.

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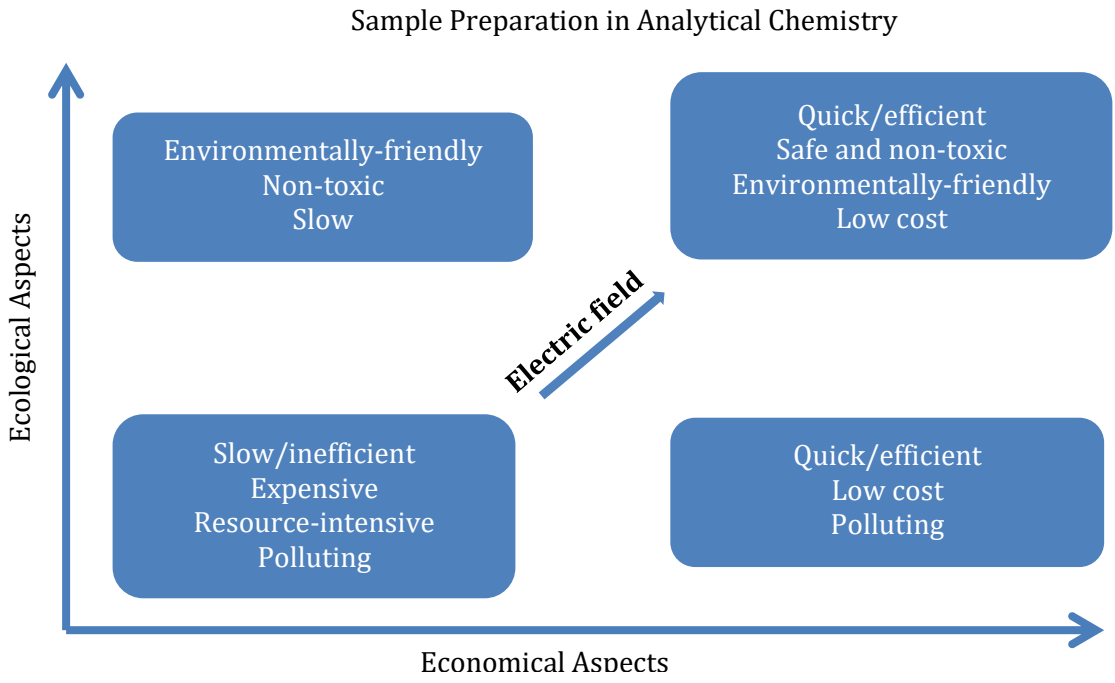
Chapter 1

The electric field – An emerging driver in sample preparation



Graphical abstract

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1.1 Abstract

An electric field can be combined with established sample preparation techniques or used as the sole driving force for sample preparation. The electric field is generally used for analyte extraction or sample purification, and in both cases this results in acceleration of the mass transfer of analytes or impurities from the sample into the acceptor phase. The sample and acceptor phases may be either in direct contact or separated by a liquid or solid membrane. This review introduces and highlights the advancements in electric field-assisted sample preparation from 2013-2015. The main sections are membrane and membrane-free approaches, including their application in the classical and microfluidic scale. The included membrane approaches are electrodialysis/ion concentration polarisation, three-phase liquid electroextraction, and electromembrane extraction. The membrane-free techniques are electric field-assisted solid-phase (micro)extraction, electrofiltration, electrophoretic concentration, and dielectrophoresis. There were 67 research articles covered and thus this is considered as an active area in analytical chemistry.

1.2 Introduction

An analytical workflow typically involves sampling, sample preparation, analytical separation, detection, and data processing and reporting. The bottleneck in this workflow is sample preparation, especially for complex samples. Nowadays, the economic and ecological aspects of sample preparation are also given importance. For the latter aspect, the twelve principles of Green Analytical Chemistry are considered.[1] The translation of these principles to sample preparation means that the use of toxic reagents and solvents should be minimised or eliminated. The purpose of many sample preparation approaches is to transfer the target analytes from the sample matrix into another phase (acceptor phase) where the analytes are trapped or even concentrated. Alternatively, the unwanted components in the sample can be transferred to the acceptor phase in order to purify the sample. In the case of analyte extraction, many research efforts have been geared towards the improvement of mass transfer from the sample matrix into the acceptor phase. These efforts have included approaches to increase the contact surface between the sample and the acceptor phase, and also the use of an auxiliary force between sample matrix and acceptor phase. A popular way to increase the contact surface is to reduce the volume of acceptor phase, such as in dispersive liquid-liquid microextraction (DLLME) [2], stir-bar sorptive extraction [3], and solid-phase microextraction (SPME) [4]. Common auxiliary drivers are pressure [5], heat, sonication [6], microwave-irradiation [7], and electric fields.

The applied electric field causes migration of charged species, as defined by the electrophoretic mobility (μ) of the species. The applied electric field also causes electroosmosis which emanates from charged surfaces. The electric field therefore affects the analyte mass transfer and also introduces selectivity to the sample preparation process. Furthermore, the electric field can be superimposed onto the sample preparation with relative simplicity; however, it is only applicable to ionised or ionisable molecules. The analytes are generally introduced in a liquid sample matrix and the acceptor phase can be a solid or liquid phase. Most

instrumental separation and detection methods deal with liquid samples and thus a liquid acceptor phase is preferred.

In this review, we highlight the advancements and potential of the use of electric fields in analytical sample preparation. The focus is on electric-field driven or assisted approaches for liquid samples in the classic and miniaturised scale. The approaches are categorised as (1) use of membranes to separate the sample and acceptor phases and (2) membrane-free approaches. In (1), relevant approaches are electrodialysis (ED)/ion concentration polarisation (ICP), three-phase liquid electroextraction (3PLE), and electromembrane extraction (EME). In (2), the relevant approaches are electric field-assisted solid-phase extraction (EFA-SPE), electrofiltration (EF), electrophoretic concentration (EC) and dielectrophoresis (DEP). EME was the most widely used technique and for this reason it is discussed in more detail.

1.3 Membrane-based approaches

A solid (i.e., in ED and ICP) or liquid (i.e., in 3PLE and EME) membrane has been used to separate the sample and acceptor solution and the purpose of this phase is to allow the transfer of analytes from the sample to the acceptor solution.

1.3.1 Use of a solid membrane to separate sample and acceptor phase

A solid membrane is used to separate the sample in ED and ICP. ED was first described for the purification of sugar syrup in the late 19th century. [8–10] ED then quickly became popular for large-scale desalination of water and later gained importance for wastewater treatment (e.g., removal of heavy metals).[11] In analytical chemistry, one or more membranes are used to separate the sample from the acceptor solution(s). Dialysis is normally performed overnight whereas ED can be completed in a few hours. When an electric field is applied, the movement of target ions through the membrane is controlled. For purification of the sample, the membrane

is permeable to the unwanted ions. For analyte extraction, the membrane is permeable to the ions of interest.

All membranes have a molecular weight (MW) cut-off and these membranes can be non-selective (NSM), anion-exchange (AEM), and cation-exchange (CEM). The membranes allow the passage of ions not exceeding a certain MW and do not allow the flow of liquid. An NSM used with an applied electric field allows the migration of all charged species and is typically made from cellulose acetate with different degrees of cross-linking. AEM and CEM used with an applied electric field allow the migration of anions and cations through the membrane, respectively. These charge-selective membranes are typically made of polystyrene cross-linked with divinylbenzene and the AEM and CEM are functionalised with either a positively-charged group (typically quaternary ammonium) or a negatively-charged group (typically sufonic acid), respectively.

In the presence of an electric field, ions move towards the relevant electrode according to their polarity and can either pass through the membrane (e.g. anions will pass through an AEM) or be unable to pass through the membrane due to electrostatic repulsion (e.g. cations will be repelled by an AEM). The ions that are allowed to pass through the membrane are removed from the sample while the retained ions are trapped on the upstream side of the membrane which is in contact with the sample. This phenomenon in close proximity to the membrane surface is referred as ICP. ICP using small membranes in microfluidic devices has resulted in very high target analyte enrichment, up to more than a million fold by ED after several hours.[12] ED in microfluidic devices is often referred to as ICP for target analyte concentration. On the other hand, ED in classical scale (mL to L sample) is mainly for clean-up, such as in desalination.

In common practice, an ED cell contains two or more membranes in a stacked membrane configuration. The sample flow-path is sandwiched between the membranes and the electrodes are placed at the anodic and cathodic sides of the cell. Electrolyte solutions are

placed at both electrode compartments and act as acceptor phases for ions transferred from the sample. An ED device can be classified in terms of the number of membranes used as single-, double- or multi-membrane devices. For single-membrane devices, either positively or negatively charged target analytes can be isolated. For double- and multi-membrane devices, both positively and negatively charged analytes can be isolated. In a double-membrane ED, two membranes are used, for example an AEM and a CEM, or two NSMs, at the anodic and cathodic sides of the cell and this configuration is generally used to purify the analytes in the sample matrix. In multi-membrane ED, four or more membranes are used, for example the sample is sandwiched between two NSMs and the sample and NSMs are then bracketed by selective membranes. In this case, charge and MW fractionation can be achieved between the outer membranes. Thus, multi-membrane ED is harnessed for analyte fractionation.

A single-membrane ED was reported for the enrichment of cationic drugs of abuse from spiked plasma samples.[13] A new CEM was made by casting of a solution of basic polymer (i.e., cellulose acetate), plasticiser (i.e., tris(2-ethylhexyl)phosphate) and cation-selective ion carrier (i.e., di-(2-ethylhexyl)phosphoric acid) over a glass capillary. The resulting tube-shaped membrane was submerged in 3 mL of sample and 20 µL of acceptor phase was placed in the lumen of the CEM. Analyte isolation was completed in 10 min at 300 V with analyte concentration factors of 97-103.

Double-membrane ED was used to completely separate dissolved organic nitrogen (DON) from dissolved inorganic nitrogen (DIN) in water samples.[14] DON determination is generally performed by subtracting the DIN (i.e., nitrate, nitrite, and ammonia) from the total nitrogen (TN). However, using a commercially-available AEM and CEM allowed the removal of DIN whereas the higher MW DON (MW> 500 Da) was retained in the sample. Figure 1.3.1.1 shows the ED cell which contained a static sample of 100 mL. On the downstream side of the membranes was a continuous flow of a 0.5 M sodium chloride solution to remove the ions which passed through the membrane. This flow was important to maintain a steep DIN concentration

gradient across the membrane. An aluminium electrode was immersed into each sodium chloride solution. A non-inert light metal was used as sacrificial anode to suppress the formation of elemental oxygen and chlorine, which can oxidise the DON. Spectroscopic analysis was used to determine the DIN and TN.

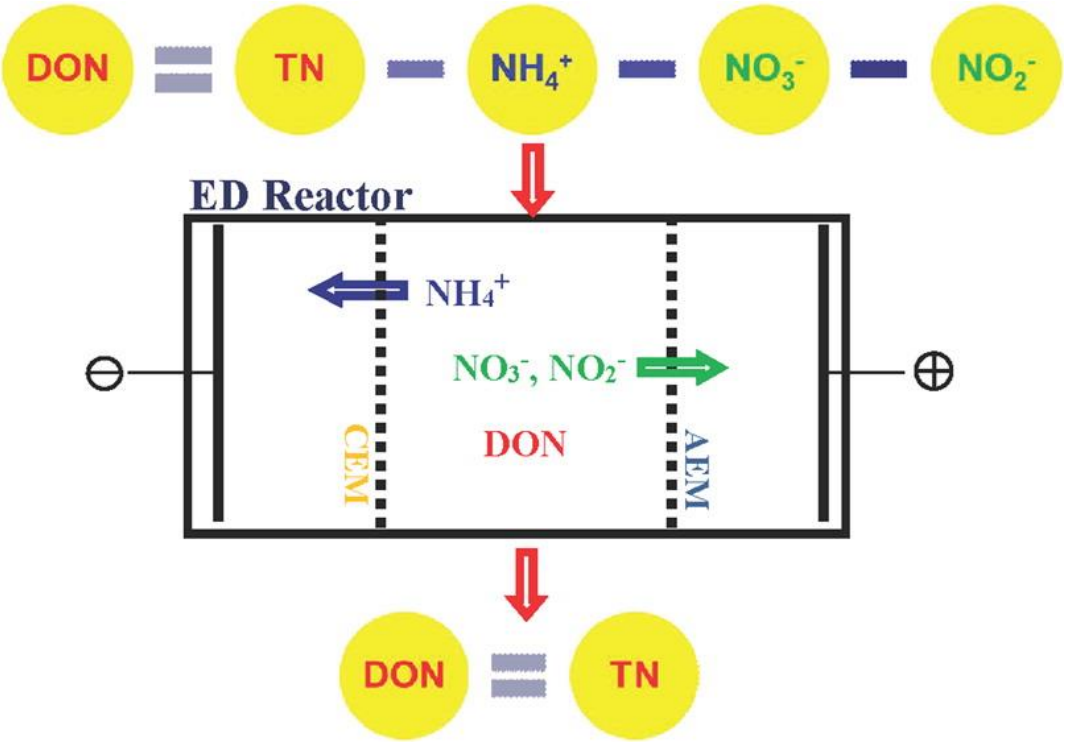

Figure 1.3.1.1 shows the electrodialysis cell for the determination of dissolved organic nitrogen (DON) in water. The cell contained three chambers which were separated by a cation-exchange membrane (CEM) and an anion-exchange membrane (AEM). The AEM and CEM were permeable for dissolved inorganic nitrogen (DIN), but not for DON. The 100 mL sample was placed in the middle chamber enclosed by the CEM and AEM. On the downstream side of the membranes was a continuous flow of a 0.5 M sodium chloride solution to remove the ions which passed through the membrane. An aluminium electrode was immersed into each sodium chloride solution. A potential of 30 V was applied between the two electrodes. Spectroscopic analysis of the sample after electrodialysis was used to determine the DON as total nitrogen (TN) after removal of DIN.

Multi-membrane ED approaches have been reported for the fractionation of various target analytes from food samples.[15,16] Two notable reports were the use of NSMs adjacent to the sample and selective membranes at the outer sides of the cell. Simultaneous fractionations of anionic and cationic peptides were performed at the anodic and cathodic sides of the four-membrane ED device. Fifteen anionic peptides with hypocholesterolemic, antihypertensive and antibacterial properties and four cationic peptides including lactokinin with antihypertensive property were fractionated.[15] The peptide mixture was from trypsin hydrolysis of β-lactoglobulin and the fractions from ED were subjected to LC-MS. Low molecular peptides (MW 300-500 Da) with the capacity to improve glucose uptake in L6 muscle cells have also been fractionated in the same way.[16] In this case, the peptide mixture was from pepsin and pancreatin hydrolysis of soy protein isolate and the fractions from ED were also subjected to LC-MS.

An interesting work using multi-membrane ED has been directed to the fractionation of cationic peptides from flaxseed protein hydrolysate.[17] The ED device had one membrane (an AEM) at the anodic side while three membranes were configured at the cathodic side of the cell. At the cathodic side, a NSM with MW cut-off of 50 kDa was adjacent to the sample. This was followed by another NSM with a MW cut-off of 20 kDa and finally a CEM. The peptide fractions were collected in the zones between the two NSMs (fraction 1) and the 20 kDa NSM and CEM (fraction 2), which were initially filled with KCl solution. Fraction 1 and 2 showed blood pressure lowering effects in hypertensive rats and increased glucose uptake in L6 muscle cells, respectively. However, the expected MW of the cationic peptides (20-50 kDa in fraction 1 and <20 kDa in fraction 2) that should be trapped in between the membranes did not correspond to the MW of the fractionated peptides reported (300-400 in fraction 1 and 400-500 in fraction 2). The ED time of six h used in this study was insufficient to allow the electrophoretic migration of the higher MW peptides.

In microfluidic devices, the membrane is fabricated directly into the device itself, typically by intersecting one or more microchannels with a suitable membrane.[18–20] A commonly used membrane has been Nafion, a perfluorinated ion-exchange material, which was patterned on the microchannel during the fabrication of the microfluidic device.[18] This membrane afforded two to three orders of magnitude concentration ratios of two model compounds, namely fluorescently-labelled DNA [19] and bovine serum albumin.[20] A membrane formed from the dielectric breakdown of polydimethylsiloxane (PDMS) was used for the double membrane ICP of fluorescently-labelled ampicillin from whole human blood.[21] Figure 1.3.1.2(a) shows the design of the device. Two V-shaped channels (denoted 2 and 4) were connected via the PDMS membrane to the straight separation channel (denoted 1). (b) is a schematic illustration of device, where the convergent sample channel (left side in (b)) was separated from the separation channel by the PDMS membrane with a MW cut-off of <1000 Da. This membrane allowed the passage of the target analytes and inorganic ions into the separation channel. A second convergent channel was connected to the separation channel (right side in (b)) by using a second PDMS membrane that permitted the passage of inorganic ions away from the separation channel where only the target analytes were concentrated.

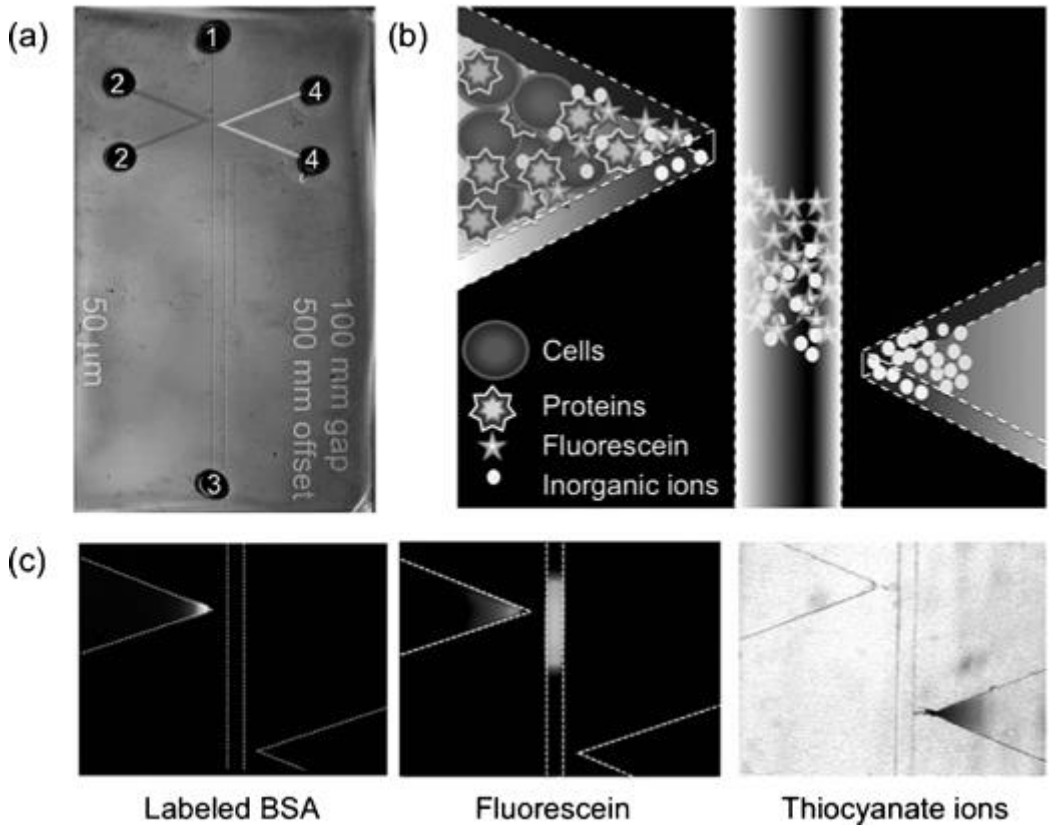

Figure 1.3.1.2 shows the microfluidic device for selective extraction of analytes from whole blood. In (a), the design of the device is presented. Two V-shaped channels (denoted 2 and 4) were connected via the PDMS membrane to the straight separation channel (denoted 1). (b) is the schematic illustration of microfluidic channel junction, where the convergent sample channel (left side in (b)) was separated from the separation channel by membrane with molecular weight cut-off of <1000 Da. This membrane allowed the passage of the target analytes and inorganic ions through the membrane into the separation channel. A second convergent channel was connected to the separation channel (right side in (b)) by using another membrane that permitted the passage of inorganic molecules away from the separation channel where only the target analytes were concentrated. (c) Three microscopy images of fluorescently-labelled bovine serum albumin which could not pass the membrane (left), fluorescein sample extracted into the separation channel (middle), and thiocyanate ions removed from the separation channel (right).

1.3.2 Use of a liquid membrane to separate sample and acceptor phase

The techniques of 3PLE and EME are used for concentration of analytes in an aqueous sample to a small volume of aqueous acceptor phase. A liquid-phase is used to separate the sample and acceptor phase in these analytical techniques. In 3PLE, the sample and acceptor phases are separated by a water-immiscible solvent. The immiscible solvent has been referred to as a free liquid membrane (FLM).[22] In EME, the water-immiscible solvent is immobilised in a

porous support. This assembly is known as a supported liquid membrane (SLM) [23] and the support is a thin (flat) film or a hollow fibre. EME was developed originally using a SLM with a hollow fibre.[23] In the case of a thin film SLM, the acceptor phase is placed on one side of the film. In the case of a hollow fibre, the acceptor phase is contained inside the lumen of the fibre. The obvious advantage of a hollow fibre over a thin film SLM is the larger surface area in contact with the sample. In this review, we use the terms FLM and SLM to refer to the liquid membrane in 3PLE and EME, respectively. Further interesting fundamental information and novel applications of EME and 3PLE were recently reviewed.[24–28]

There were two configurations performed in 3PLE. In the first configuration which was also referred to as micro-EME, the FLM was situated between the sample and acceptor phases which were inside a tube (i.e., polypropylene or perfluoroalkoxy tube).[22,29,30] 0.5-2.0 μL of deionised water or 10 mM acetic acid were used as acceptor phases while ethyl-2-nitrobenzene, 2-nitrophenyloctylether, 1-pentanol, or 1-hexanol were used as FLMs. The analytes were cationic and anionic dyes, perchlorate, and positively charged basic drugs which were electroextracted from different sample matrices (i.e., water, urine and blood serum). When the volume of the acceptor phase was 100x smaller than the sample, analyte concentration factors of 15-98 were obtained.[29] In addition, electrolysis was found to have a negative effect on the electroextraction using low capacity buffered or unbuffered acceptor phases.[30] In the second configuration, a drop of the acceptor phase (i.e., 5% formic acid in 33% methanol) was submerged into the FLM (i.e., ethylacetate:methylacetate, 3:2, v:v) using a conductive pipette tip.[31] Carnitine and several of its derivatives in 50 μL of spiked human plasma were electroextracted into 2 μL of the acceptor phase. Small concentration factors (<7) were reported and 3PLE with this single drop FLM was suggested for purification in bioanalysis with mass spectrometric analysis.

In EME, thin film SLMs using mainly commercially-available polypropylene-based membranes were reported during this review period.[32–36] The SLMs were used successfully for

the exhaustive extraction of peptides and basic drugs from purified water and human plasma [32,33], and food dyes in purified water [34]. Figure 1.3.2.1 shows a two-step EME for selective isolation of angiotensin II antipeptide from a peptide mixture in buffer solution.[35] The first step (step #1: Extraction) was electroextraction of all peptides into the acceptor solution. In the second step (step #2: Clean-up), the pH of the acceptor solution was adjusted to the isoelectric point of the target peptide and the polarity was reversed. This caused the migration of the charged peptides out of the acceptor electrolyte and only the target peptide remained in the acceptor solution. EME was combined with liquid-phase microextraction (LPME), where EME and LPME were used for the extraction of basic and acidic drugs, respectively, from human plasma.[36] EME was introduced for high throughput sample preparation on a 96-well plate format.[37] This allowed extraction of three cationic drugs simultaneously from human plasma and urine samples within 10 min. In addition, a novel agar film silver nanoparticle-based SLM was compared with commercially-available polypropylene membranes. The former SLM showed 10 to 70-fold higher extraction efficiency.[38]

The use of a hollow fibre SLM was the most commonly used approach for EME.[39–59] The most prevalent hollow fibre was made from polypropylene. A typical liquid phase immobilised in the pores of the hollow fibre was 2-nitrophenyloctylether due its desirable characteristics, including low water solubility, high dipole moment, high proton acceptor properties, and low proton donor properties.[39]

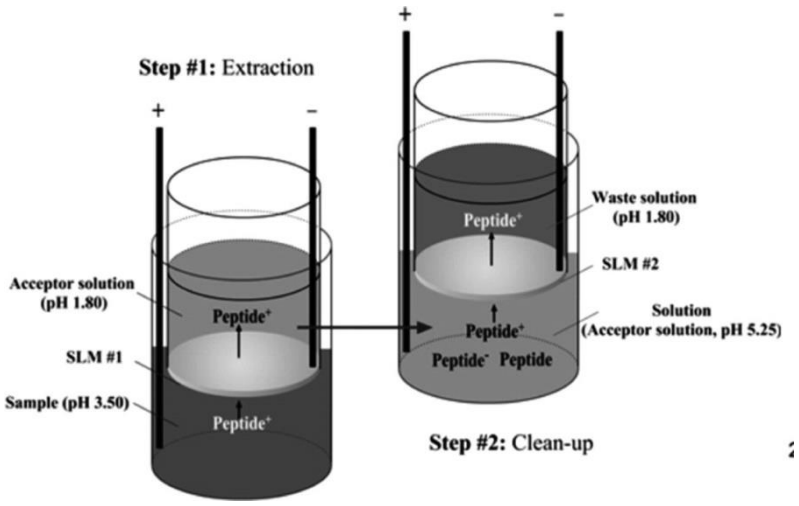

Figure 1.3.2.1 shows the two-step EME and selective isolation of angiotensin II antipeptide from a peptide mixture consisting of angiotensin II, neurotensin, angiotensin I and leu-enkephalin. In the first step, EME was performed to transfer the net-positively charged peptides (denoted Peptide$^+$) from the sample to the acceptor phase. The neutral and negatively-charged sample constituents remained in the sample. In the second step, the pH of the acceptor phase was adjusted to the isoelectric point of the target peptide and the polarity was reversed. This caused the migration of the charged non-target peptides (denoted Peptide$^+$) out of the acceptor electrolyte and only the neutral target peptide (denoted Peptide) remained in the acceptor solution.

In single hollow fibre EME, either positively or negatively charged analytes have been extracted.[39–57] The hollow fibre which contained the acceptor electrolyte was dipped in the sample and the electrodes were placed in the acceptor phase and the sample. The analytes were inorganic ions and small molecules, such as drugs, metabolites and peptides. The samples were from biological (i.e., blood, plasma, urine, saliva, breast milk), environmental (i.e., wastewater, drinking water, cigarette smoke) and food (i.e., gelatine) sources.

In order to improve analyte enrichment and/or extraction speed, modifiers (e.g., added to the sample), the hollow fibre SLM, EME electrode configurations, and voltage pulsing have been investigated. Modifiers added to the liquid phase of the SLM included ionic liquids [40,41], 18-crown-6 ethers [42], silver nanoparticles [43], or carbon nanotubes [44]. Modifiers added to the sample solution included anionic and non-ionic surfactants.[45,46] Innovative hollow fibres, such as a

wheat stem instead of the typical polypropylene hollow fibre (e.g. internal diameter of 600 μm, wall thickness of 200 μm and a pore size of 0.2 μm) have also been investigated.[47]

A nano-polypropylene tube as SLM support has been implemented on-line with capillary zone electrophoresis (CZE).[48] The nanotube was used to envelope a crack which was made 2 cm away from the inlet end of a fused-silica capillary. The crack was necessary to allow the entrance of ions from the sample to the capillary during voltage application. The nanotube and capillary inlet was placed in the sample (i.e. cationic drugs in 10 mM HCl) and CZE separation buffer (i.e., 60 mM sodium phosphate buffer at pH 2.7), respectively. During EME, a voltage was applied across the sample and inlet buffer vials in order to introduce the drugs into the acceptor phase, which in this case was the separation buffer. This is reminiscent of the stacking technique of field-enhanced sample injection (FESI) used in capillary electrophoresis (CE) where the analytes are injected electrokinetically from a low conductivity sample into a high conductivity separation buffer.[60] The organic liquid phase in the SLM could also have contributed to the improvement in FESI. In fact, it has been found that the electric field strength was enhanced by the organic solvent in the SLM.[49] The CZE separation was performed by applying voltage between the short capillary and the separation capillary. Up to 500-fold improvement in on-line UV-detection sensitivity was achieved in 5 min of EME with FESI across the short capillary.

The classical EME electrodes consist of two straight wires. Theoretical studies on the electric field profiles of three electrodes indicated higher field strengths for EME using helical or cylindrical electrodes.[49,50] However, experimental results showed a better performance with the classical electrode. Voltage pulsing during EME has been used to overcome extraction instability caused by Joule heating.[51–55] The voltage was switched on for 3-30 s and off for 2-15 s order to allow dissipation of heat between pulses.

In addition, single hollow fibre EME has been combined with another extraction technique (i.e., SPME).[56,57] The common configuration of EME using aqueous sample and

acceptor phase that are separated by a SLM made of a polypropylene hollow fibre with a water-immiscible solvent or solvent mixture was used. A carbon-based electrode was dipped in the acceptor phase and this electrode was used as the sorbent for SPME. There is potential for this dual extraction approach for sample purification of complex samples.

Two hollow fibre SLMs were used for simultaneous extraction of cationic and anionic analytes.[58,59] The fibres were dipped into the sample and the electrodes were placed in the acceptor phases. The cationic and anionic analytes were thebaine and ibuprofen [58] or chromium (III) and chromium (VI) [59]. The samples were water, plasma and urine. The reported extraction recoveries were lower than single hollow fibre EME, but this was because a compromise in the extraction conditions had to be made in order to allow the ionisation of all analytes and sufficient extraction time for both analyte species.

1.4 Membrane-free approaches

In membrane-free approaches, the sample is in direct contact with a solid (i.e., EFA-SPME, EF) or liquid (i.e., EC) acceptor phase, or no acceptor phase is involved (i.e., DEP). DEP is only suitable for particle separation from liquid solution.

1.4.1 Electric field-assisted solid-phase extraction

The idea of separating analytes using an electric field in combination with a solid acceptor phase was introduced in 1963 and termed as electrochemically modulated liquid chromatography.[61] The electric field was used to manipulate the surface of the solid acceptor and also affect absorption/desorption of analytes between the solid acceptor and elution solvent.[4,62–64] In both EFA-SPE and EFA-SPME, the sample was loaded onto the solid acceptor and the retained analytes were then eluted with a suitable solvent. In EFA-SPE, the sample was loaded into a cartridge that contained the acceptor. Before chemical analysis, evaporation of the elution solvent, followed by reconstitution with another suitable solvent was then applied. In

EFA-SPME, the solid acceptor phase is a conductive fibre which was submerged in the sample. Prior to chemical analysis, solvent elution or thermal desorption from the acceptor was performed. These approaches showed faster extraction times and better sensitivity with analyte enrichments of up to a factor of 159 in comparison with classical SPE/SPME.

EFA-SPE was performed off-line for cationic marbofloxacin from milk [65] and anionic dye sunset yellow in buffer solution [66]. In both studies, the solid acceptor was a commercially-available silica material and the electrodes were placed above and below the solid sorbent. After loading the sample on top the SPE sorbent, the analytes were eluted in the presence of an electric field to enhance the recovery.

EFA-SPME was performed off-line for methamphetamine [67] and proline [68] in urine, and tricyclic antidepressants in environmental waters [69]. The solid acceptor phases were synthesised polypyrrole [68] and commercially-available fibres [67,69]. A Pt-wire and the SPME fibre served as the electrodes submerged in the sample. The analytes were extracted from the sample by voltage with the SPME fibre as cathode. For proline, 0.1 M NaCl was used for desorption with the Pt-wire as cathode. For methamphetamine and tricyclic antidepressants, the SPME fibre was transferred to the GC-MS for direct thermal desorption of analytes.

EFA-SPME was proposed at-line with HPLC for the determination of naproxen in urine [70] and diclofenac in urine and environmental waters [71]. In both assays, the solid acceptor phase inside a metal tube was electropolymerised polypyrrole, which acted as an anion-exchanger. The anionic analytes were loaded into the SPME device followed by washing with water. A 10 mM phosphate buffer at pH 3 mixed with acetonitrile (1:1 v/v) was then loaded into the device and voltage was applied with the anode at the HPLC side of the device. Sample injection was performed by switching the HPLC-valve to direct the liquid inside the SPME device to the analytical HPLC column. The role of the electric field was not clear since the final solution inside the SPME device during voltage application neutralised the charge of the acidic analytes. The release of the analytes was perhaps due to the weakening of the interaction between the anion-

exchanger and the anionic analytes at low pH, although application of the electric field was reported to provide better sensitivity compared to no voltage application.

1.4.2 Electrofiltration/electromicrofiltration

EF or electromicrofiltration (EMF) was mainly used for water purification where the sample was fed through a filter by pressure. The electric field was used to prevent blocking and fouling of the filter by electromigration of the unwanted components away from the filter. The most notable developments for water purification were on filter materials that selectively remove contaminants. Polymeric materials, such as polyvinylidene fluoride and Magnéli Ti_4O_7 modified ceramic membrane, were used to remove humic substances, calcium, benzophenone-3, and bovine serum albumin.[72–74]

1.4.3 Electrophoretic concentration

In EC, a small volume of aqueous acceptor phase (i.e., 20 μL) was placed in direct contact with a liquid sample (i.e., 20 mL). The acceptor phase has a higher conductivity than the sample in order to achieve field-enhancement conditions described in CE.[60] For the EC of either cations or anions, the setup consisted of a micropipette (e.g., length = 6.4cm) containing the acceptor phase, a polyacrylamide hydrogel in a tube, two Pt-electrodes, and high voltage supply (HV). The hydrogel was fortified with buffer electrolyte to support electrophoresis and also to prevent the flow of acceptor phase out of the pipette, especially as a result of electroosmotic flow caused by the electric field.[75] One end of the micropipette was dipped into the sample while the other end was inserted into the hydrogel. The Pt-electrodes were connected to the HV supply and inserted into the hydrogel and sample solution. Stirring was applied during voltage application (0.4-1.3 kV). The cationic analytes were extracted with the cathode placed in the hydrogel, whereas extraction of anionic analytes was accomplished with the anode in the hydrogel. EC was applied to anionic pollutants and cationic drugs in different types of water samples.[76,77] The analytes (with or without preliminary sample dilution with water to lower the

conductivity of the sample) were enriched from 12 to 249 times. The acceptor phase after concentration was analysed directly using CE.

In order to simultaneously concentrate both anions and cations, two micropipettes attached to two separate hydrogels, which were connected to an anode or cathode, were dipped into the sample (see Figure 1.4.3.1). In (a), the situation before voltage application is shown where the sample contained charged analytes. In (b), extraction voltage was applied and the anions and cations migrated into the micropipettes and were separated by their charge. After simultaneous EC and separation (SECS), the acceptor phases were transferred for chemical analysis. SECS was applied for cationic quaternary ammonium and anionic phenoxypropionic acid herbicides in purified and river water samples. Analyte enrichments of up to 337 for a 30 min voltage application (i.e., 0.5-2.0 kV) were obtained.[78]

In EC and SECS, the electric field is the solely driver for transferring the analyte to the acceptor phase. The selectivity of these two approaches is dictated by the analyte's electrophoretic mobility where analytes with high and low values migrate at faster and slower velocities, respectively. The analyte enrichment relies on the conductivity ratio of sample to acceptor phase where the enrichment is better for low conductivity samples. This suggests that the two approaches are more effective for samples of low complexity and small ionised or ionisable analytes of high polarity. For samples with a high conductivity and complexity, the analyte enrichment might be reduced and biased by the presence of small and fast migrating ions (e.g., inorganic cations and anions).

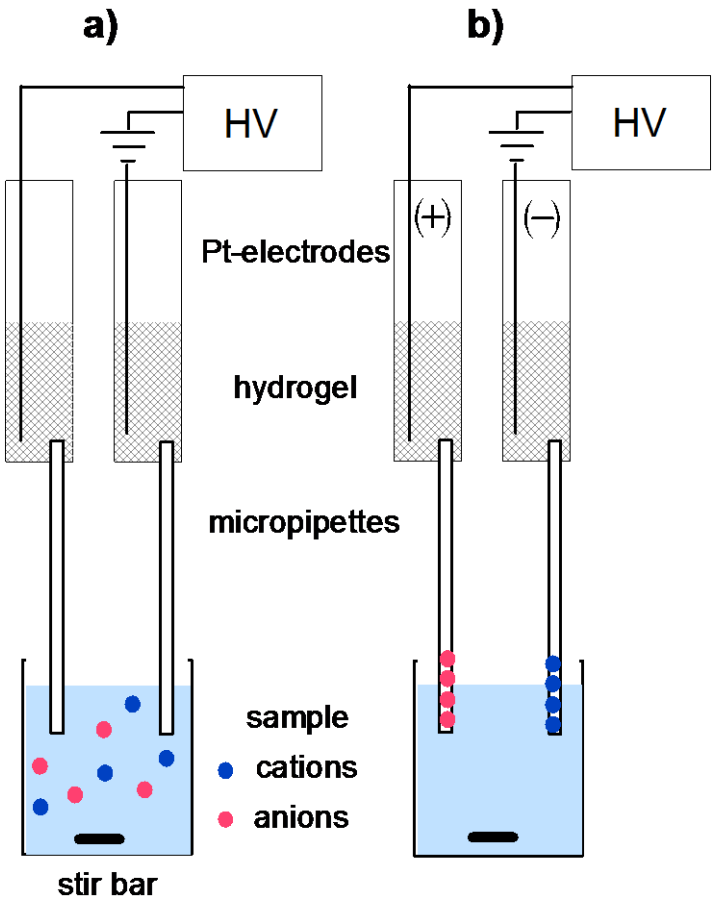

Figure 1.4.3.1 shows a schematic of electrophoretic concentration (EC) for the simultaneous EC and separation (SECS) of negatively and positively charged analytes. In (a), the situation before voltage application is shown. The acceptor phases are suspended into two micropipettes using a conductive polyacrylamide-based hydrogel. The hydrogels were connected to a high voltage supply (HV) via two Pt-electrodes. The micropipettes were dipped into the sample. In (b), extraction voltage was applied. The anionic and cationic analytes migrated into the micropipettes with the positive and negative polarity, respectively. After SECS, the acceptor phases containing the target-analytes were analysed directly.

1.4.4 Dielectrophoresis

DEP is the net movement of a polarisable particle subjected to a non-uniform electric field which is created by applying an AC voltage.[79,80] It has been used in microfluidic systems to isolate and concentrate particles based on size and dielectric properties. DEP was applied to pathogenic micro-organisms (i.e., E. coli bacteria, bacteriophage virus particles), ovarian cancer cells, fluorescing microspheres, human mesenchymal stem cells, osteoblasts, circulating tumour

cells, proteins, polystyrene particles, and yeast cells.[81–88] High particle enrichments were reported with up to five orders of magnitude within 2 h.[87] A notable application of DEP in a three compartment device was for purification of blood.[88] In the first compartment, mixing of the blood sample with antibody-coated nanoparticles against highly abundant proteins was performed. In the second compartment, the blood cells and antibody-bound highly abundant proteins were trapped by DEP and only the serum including the target proteins could pass into the third compartment where the target proteins were spatially orientated and immobilized.

1.5 Conclusion

The application of an electric field in sample preparation techniques is an emerging area in analytical chemistry. The electric field is commonly used to accelerate sample preparation, as well as to provide additional selectivity. Electric fields have been used in combination with well-known sample preparation techniques, such as dialysis, liquid extraction, and solid-phase extraction, in order to provide additional selectivity. The techniques of ED/ICP, EME, 3PLE, EFA-SPE, EC, EF, and DEP have been used for the sample preparation of a wide range of charged analytes, including inorganic ions, drugs, metabolites, peptides, and proteins. ED, EME, 3PLE, EFA-SPE, EF and EC were prevalently used on a classical scale while ICP and DEP have been mostly implemented in microfluidic devices. Strategies for analyte enrichment have been based on ICP, EME, 3PLE, EFA-SPE, EC and DEP. Very high enrichment factors of up to 10^5 to 10^8 were obtained for the microfluidic approaches of ICP and DEP. These high enrichment factors were achieved because the target molecules were trapped from a constant flow of sample. However, ICP and DEP were difficult to control and the mobilisation of the concentrated analytes was still a major challenge. The small dimensions of microfluidic devices also restricted the choice of sample preparation techniques. For the future, it is expected that the control over ICP and DEP will improve. EME, 3PLE, EFA-SPE and EC have resulted in concentration factors of 10^1 to 10^3. The limitation in analyte enrichment in these techniques results from the volume ratio of

sample phase to acceptor phase, which normally is from mL-samples to µL-acceptor volumes. A decrease of the acceptor volume is difficult since the handling of sub-µL volumes is prone to errors and would adversely affect the method's reliability. In addition, extraction instabilities caused by adverse effects from voltage application (e.g. Joule heating, electrolysis) are compromising the performance of these techniques. A better understanding of the electric field-assisted sample preparations and novel operational modes (e.g., pulsed voltage application in EME) will help to reduce the undesired effects. ED and EF were mainly used for sample purification. EME experienced a significant advancement in the current review period. This technique is also promising to be a real alternative to traditional extraction methods on a classical as well as on a microfluidic scale.

The use of purely electric field-driven sample preparation techniques has recently been demonstrated in the techniques of EC and SECS with the aid of field enhancement, which is a known electrophoretic stacking technique. There are other electrophoretic stacking techniques that may be utilised for sample preparation in the future. Finally, the efforts presented here were also geared towards green analytical chemistry. Indeed, the use of organic solvents has been minimised in all the techniques except EFA-SPE. The demand for more efficient sample preparation methods will further stimulate the study of electric field-driven techniques in the future.

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Chapter 2

Zero net-flow in capillary electrophoresis using acrylamide based hydrogel

2.1 Abstract

Zero net-flow was observed when acrylamide based hydrogel was used in a vial at one end of a fused-silica capillary during electrophoresis with electroosmotic flow. We demonstrate the detection of anionic compounds with the anode at the detector end and the field-enhanced sample injection of anionic small molecule drugs in counter-electroosmotic flow capillary zone electrophoresis.

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2.2 Introduction

The electroosmotic flow (EOF) is of fundamental importance, especially to electromigration techniques in capillaries[1–4] or microchips[5,6]. The EOF in fused-silica capillaries is provided by accumulation of cations at ionized silanol groups at the inner capillary wall. Under an applied electrical field, these cations migrate to the cathode and propel water from the bulk solution with a characteristic plug flow profile. In electromigration techniques, the EOF is typically controlled to improve on separation[7–16] and more recently on stacking or on-line sample concentration[17–25]. A common approach to control the EOF is by modifications of the chemical groups at the surface of the capillary wall by using dynamic or semi-permanent[7–13] and permanent[14–16] coatings.

Hydrogels are formed from the swelling of hydrophilic polymer networks due to the penetration of water into the network. The chemical and physical crosslinking prevents the polymers from dissolving while maintaining a high water content in the polymer structure. Hydrogels can be made to hold electrolytes by simply mixing the appropriate buffer with the monomers, crosslinker, and initiator before polymerization. In capillary electrophoresis (CE), a hydrogel coating has been used to manipulate the EOF.[26] A hydrogel which exhibits EOF was demonstrated for separation and biocatalytic applications that require passage of a solvent stream through the gel.[27] A hydrogel was used to encapsulate an enzyme for use as an integrated on-line enzyme reactor CE system.[28] Hydrogels were also simply added into the separation buffer as a dynamic modifier to reduce analyte adsorption effects and to enhance reproducibility and separation.[29-31] In microfluidic devices, hydrogels have been used mainly for preconcentration or enrichment[32-35] and also for separation[36]. In addition, a DNA-containing hydrogel plug immobilized in a microfluidic channel was demonstrated as a diagnostic microfluidic assay device via electrophoresis with a sacrificial fluorescent-tagged indicator oligomer.[37]

Closing one or both ends of the capillary is a possible approach to control the bulk flow inside the capillary. However, there are technical difficulties in closing the ends of the capillary, which could potentially prevent the electrical connectivity between the electrolytes in and outside of the capillary where the electrodes are located for electrophoresis. While this paper was being reviewed elsewhere, Oucacine and Taverna reported the use of 22% polyethylene oxide gel or viscous solution in a vial at one end of a capillary for the reduction of the apparent fluid flow in capillary isotachophoresis (cITP).[38]

Although hydrogels are mechanically strong, they also are soft and elastic. Thus, polyimide-coated capillaries and electrodes used in CE can easily penetrate hydrogels. In this work, hydrogels were made directly in CE vials by thermal polymerization of an aqueous mixture of acrylamide, *N,N*-dimethylacrylamide, potassium persulfate (initiator), and common electrolytes (i.e., sodium phosphate buffer). The hydrogel filled vial was used in lieu of the electrolyte filled vial in CE. The hydrogel physically blocked the anodic end of the capillary. This prevented the bulk liquid flow due to electroosmosis whilst maintaining electrical connectivity since the hydrogel can carry electrolytes in its water rich structure. The phenomenon of zero net-flow by acrylamide based hydrogel was demonstrated by the reversal of the electrophoretic migration order of anionic compounds in capillary zone electrophoresis (CZE) and the field-enhanced sample injection (FESI) of anionic small molecule drugs in counter-EOF CZE. In addition, the effect of hydrogel composition and CE electrolyte pH were studied.

2.3 Materials and methods

Hydrogels were prepared directly in the 2 mL capacity CE vials by mixing 55%-wt acrylamide (monomer), 99% *N,N*-dimethylacrylamide (co-monomer), electrolyte stock solution, purified water, and 5%-wt potassium persulfate (initiator). The ratio of monomer, crosslinker and initiator was 15:1:1.5. The other ratios are described in the text. The total volume was 1.2

mL. Aliquots of electrolyte stock solutions (e.g., 250 mM phosphate buffer, pH 7.4) and purified water were added to make up a final concentration of the total hydrogel volume similar to that of the separation or background electrolyte (BGE). The polymerization was thermally initiated at 60 ^{o}C for 10 min. To remove excess reagents after polymerization, a small volume (e.g., 100 μL) of separation solution was placed into the vial and then was removed.

The CE experiments were performed on a Beckman P/ACE MDQ (Beckman-Coulter, USA) equipped with UV detector (214 nm) and 50 μm i.d. fused-silica capillary of 60 cm total and 50 cm effective length, respectively. The capillary was thermostated at 20 ^{o}C. New capillaries were conditioned by flushing (2 bar) with 0.1 N NaOH (30 min), followed by purified water (10 min), and separation electrolyte (10 min). Before each run, the capillary was flushed at 2 bar with 0.1 N NaOH (1 min), followed by purified water (1 min), and separation solution (5 min). All the steps described above were carried out without the placement of a hydrogel at the anodic end of the capillary. The experiments with hydrogel were performed without modification of the CE instrument. The CE procedure was basically the same without (see Figure 2.3.1A) or with (see Figure 2.3.1B) the use of hydrogel. The capillary was inserted directly into the vial containing hydrogel, as with regular CE. After each experiment with hydrogel, the capillary end was wiped with a damp tissue. The hydrogel vial can be used to as much as 5 CE runs. In the calculation of EOF, electrophoretic, and apparent electrophoretic velocities, the sign is negative and positive if electrophoresis was performed with anode and cathode at the detector end, respectively. The peak width was according to the method used by the United States Pharmacopeia (USP). The corrected peak widths were calculated by dividing the peak width by the peak migration time. The LOD was estimated at a signal to noise ratio (S/N) of 3 based on peak height.

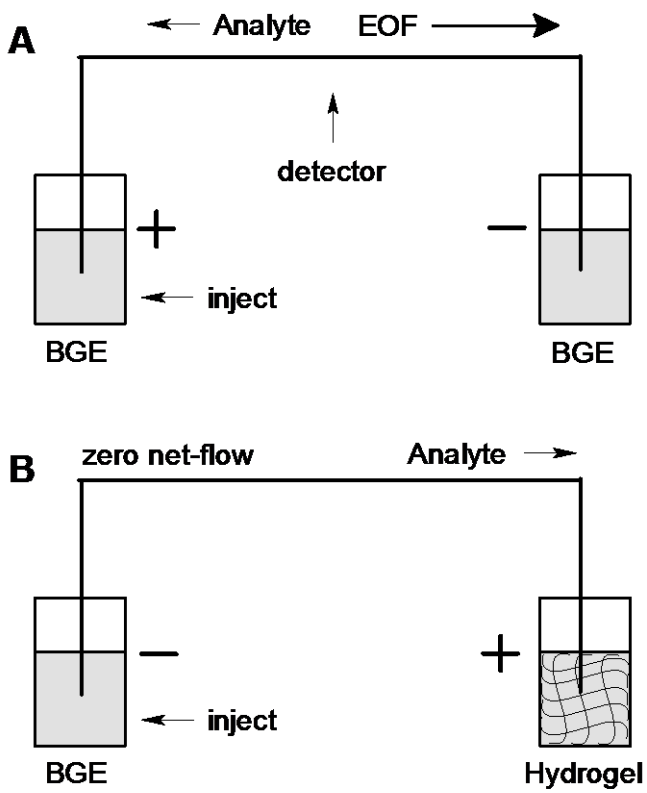

Figure 2.3.1. Schematic of normal counter-EOF CZE (A) and CZE with hydrogel at the anodic or outlet end of the capillary (B).

2.4 Results and discussion

The counter-EOF CZE of pravastatin, indoprofen and tolfenamic acid using 100 mM phosphate at pH 7.4 and 20kV (normal polarity) as electrolyte and applied voltage, respectively is shown in Figure 2.4.1A. Sample solution was 150 µg/mL of pravastatin (peak 1), 50 µg/mL indoprofen (peak 2), and 50 µg/mL tolfenamic acid (peak 3) prepared in the separation electrolyte. Injection was at 50 mbar for 5 s with separation electrolyte at the capillary outlet end. The EOF velocity > electrophoretic velocity of the analytes, and thus the analytes were detected with the cathode at the detector end. The (apparent) EOF velocity was 5.45 cm/min while the electrophoretic velocity of pravastatin (peak 1), indoprofen (peak 2) and tolfenamic acid (peak 3) was -2.19, -3.00, -3.43 cm/min, respectively. The electrophoretic mobility of the EOF, peak 1, 2, and 3 was 1.64 x 10^{-2}, -0.66 x 10^{-2}, -0.90 x 10^{-2}, and -1.03 x 10^{-2} $cm^2/V*min$,

respectively. The analytes were detected in the order of increasing electrophoretic mobility. The mobility was positive and negative if it was directed to the cathode and anode, respectively. The %RSD (n=3) for all the electrophoretic velocity and mobility values reported here were less than 0.5%.

Figure 2.4.1B shows the same CZE experiment in Figure 2.4.1A but with the hydrogel filled vial at the anodic and detector end of the capillary. The capillary was conditioned with the separation electrolyte but after sample injection, a reversed polarity at -20kV for separation was used. The electropherograms in the Figure 2.4.1 were drawn in the same scale.

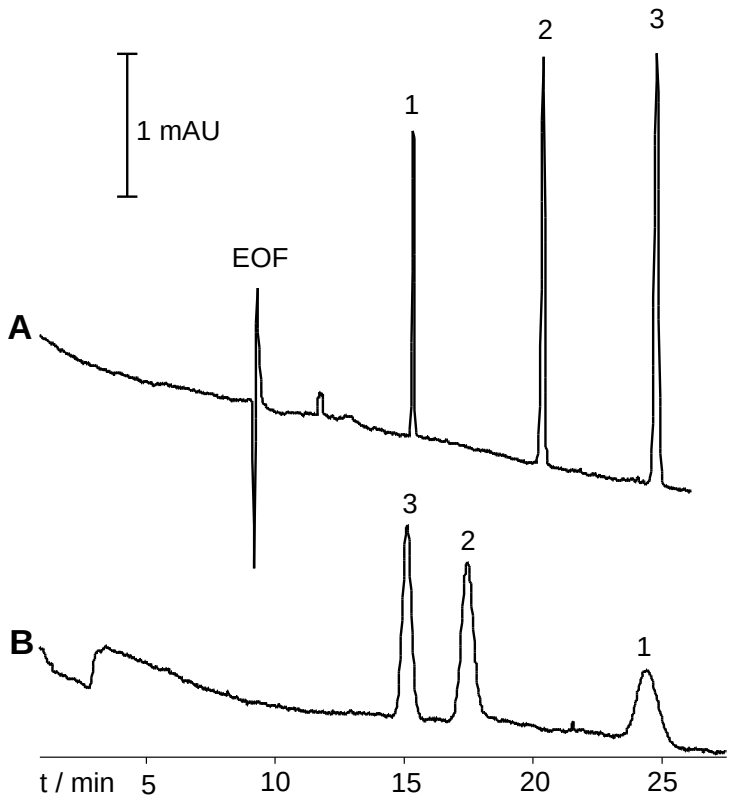

Figure 2.4.1. Normal counter-EOF CZE (A) and CZE with hydrogel at the anodic or outlet end of the capillary (B). All electropherograms were drawn in the same scale and the inserted bar indicates the absorption value. The conditions are found in the text.

The migration order in counter-EOF CZE reversed in B where the analytes were detected in the order of decreasing electrophoretic mobility (peak 3, 2, and then 1). In B, the apparent electrophoretic velocity of pravastatin, indoprofen, and tolfenamic acid was -2.05, -2.87, and -3.32 cm/min, respectively. These values corresponded very well to the electrophoretic velocity obtained in the counter-EOF CZE (Figure 2.4.1A). The electrophoretic mobility of peaks 1, 2 and 3 did not change (-0.62×10^{-2}, -0.86×10^{-2}, and -1.00×10^{-2} $cm^2/V*min$, respectively). The net-flow was then approximately zero in Figure 2.4.1B or the EOF was cancelled as a result of the hydrogel. The magnitude of the hydrodynamic flow from blocking the end of the capillary is equal to the EOF, thus the net-flow was zero.[39] The gel-to-gel repeatability was briefly assessed. The %RSD (n = 4) for migration time, peak height and corrected peak area (corrected peak area = peak area/migration time) was 0.1 – 0.2%, 3.8 – 4.5%, 1.1 – 6.6%, respectively.

The effect of the hydrogel solidity on the net-flow was studied by the amount of monomer solution added in the hydrogel preparation. Eight hydrogels were prepared with 0.1 to 0.8 mL monomer solution (55 %-wt. acrylamide in water). The total volume of the hydrogel remained constant at 1.2 mL through compensation with the added amount of water. The hydrogel was a viscous liquid when 0.1 to 0.3 mL monomer solution was used. The hydrogel was solid when 0.4 to 0.8 mL was added. Using the same conditions as in Figure 2.4.1B, zero net-flow was only achieved with solid hydrogels.

However, there was peak broadening with the use of a hydrogel in Figure 2.4.1B due to the hydrodynamic flow generated by the blocking of the capillary. A circulating EOF that caused a pressure-induced or hydrodynamic back-flow in the middle of the channel.[39] Sharp peaks were, however, obtained by Oukacine and Taverna due to the focusing nature of cITP.[38] The extent of broadening or broadening factor was calculated by dividing the corrected peak widths obtained with the use of hydrogel by the corresponding widths without the hydrogel. A high value suggests stronger peak broadening due to the hydrogel. The broadening factor was 3.8,

2.7, and 2.4 for pravastatin, indoprofen and tolfenamic acid, respectively. The broadening caused a 50 to 75% decrease in peak height with the hydrogel.

It is well known that the EOF velocity increases with the increase in electrolyte pH. Small inorganic anions (bromide, nitrate, and bromate) with electrophoretic mobilities that are not significantly affected by changes in pH were analysed by counter-EOF CZE. The results at pH 3.0, 7.4, and 9.9 are shown in Figure 2.4.2A, B, and C, respectively. Sample solution was 100 μg/mL of bromide (peak 1), 40 μg/mL nitrate (peak 2), and 400 μg/mL bromate (peak 3) in separation electrolyte. The sample was injected from the short end (10 cm to the detector) or cathodic end at 5 kV for 5s. Sample injection was performed without the use of a hydrogel filled vial. Voltage (-20 kV) was applied at reversed polarity because the electrophoretic velocities of the anions were faster than the EOF velocity. The EOF velocity was 2.08, 5.45, 6.54 cm/min at pH 3.0, 7.4, and 9.9, respectively. The EOF mobility was 0.62×10^{-2}, 1.64×10^{-2}, and 1.96×10^{-2} $cm^2/V*min$, correspondingly. The corresponding CZE with hydrogel in the anodic end is shown in Figure 2.4.2D, 2.4.2E, and 2.4.2F. The migration times decreased due to the zero net-flow. More importantly in Figure 2.4.2D, 2.4.2E, and 2.4.2F, the hydrogel consistently produced a zero net-flow at the studied pH range as indicated by the similar migration times for the analytes. The broadening factors were calculated as described above and the values were between 1.0 and 1.3. We also observed a 0 to 50% decrease in peak height with the hydrogel. These numbers are better than those obtained for the anionic drugs in the previous section. The decrease in peak height with the increase in pH of the separation electrolyte was probably due to the strength of the hydrodynamic flow from blocking the capillary with hydrogel. This flow was believed to be directly proportional to the EOF velocity. A more detailed study on the hydrogel induced peak broadening by Taylor dispersion analysis will be performed in the future.

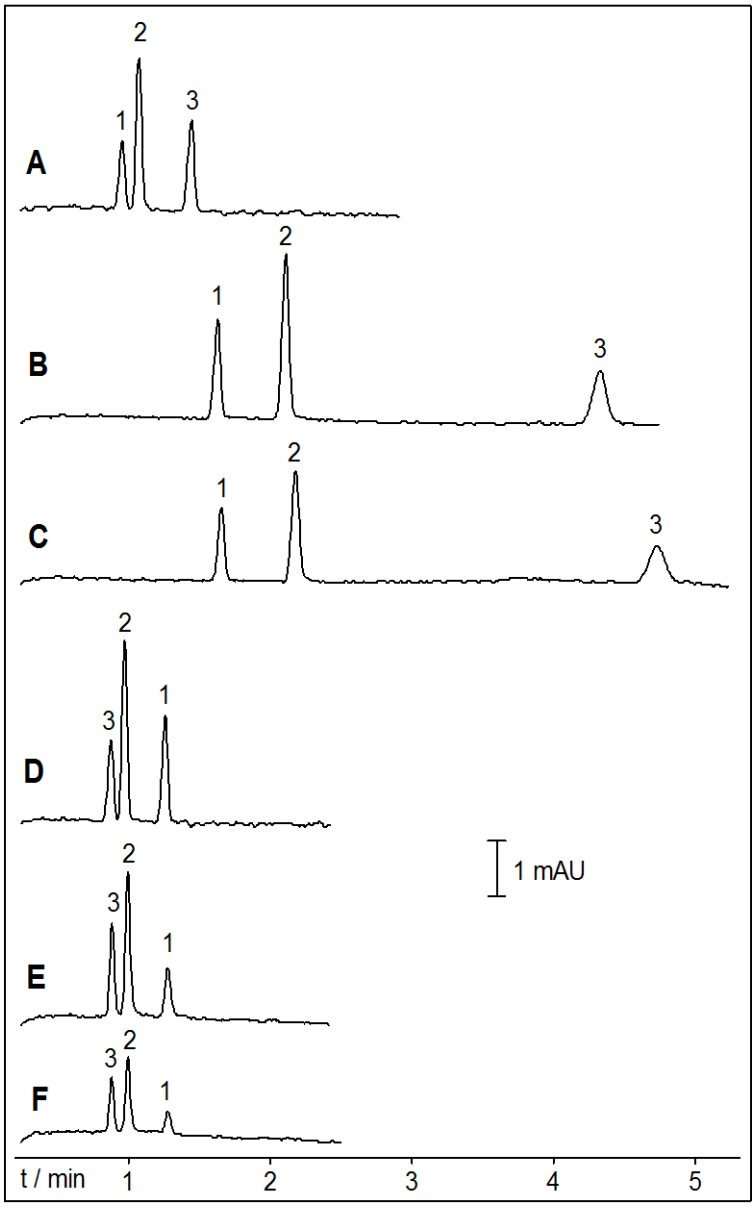

Figure 2.4.2. Effect of EOF velocity by manipulation of pH on the CZE of small inorganic anions with hydrogel. Separation electrolyte was 100 mM sodium phosphate at pH 3.0 (A, D), 7.4 (B, E), and 9.9 (C, F). In (A-C), vials containing separation electrolyte were placed at both ends of the capillary. In (D-F), a hydrogel was placed at the anodic end of the capillary and separation electrolyte at the cathodic end. All electropherograms were drawn in the same scale and the inserted bar indicates the absorption value. The conditions are found in the text.

The repeatability of migration time, corrected peak area and peak height were calculated for the experiments using a hydrogel in Figure 2.4.2D – F. At pH 3.0 (Figure 2.4.2D), the %RSDs (n=3) were 0.2 – 0.3%, 5.7 – 9.3%, and 3.2 – 8.4%, respectively. At pH 7.4 (Figure 2.4.2E), the %RSDs (n=3) were 0%, 8.0 – 12.3%, and 7.3 – 10.3%. At pH 9.9 (Figure 2.4.2F), the

%RSDs (n=3) were 0 – 0.4%, 2.7 – 10.1%, and 1.8 – 3.0%, correspondingly. The USP resolution for peaks 1 and 2 and peaks 2 and 3 were calculated. The values at pH 3.0, 7.4 and 9.9 were 1.4 and 4.2, 1.8 and 3.9, and 1.9 and 4.1, respectively. These values were similar to those obtained from experiments without hydrogel. The USP plate numbers for peaks 1, 2 and 3 at pH 3.0 were 2448, 3457, and 4639, respectively. The numbers at pH 7.4 and pH 9.9 were 3210, 3681, and 4100, and 3569, 4212, and 4474, correspondingly. The plate numbers without hydrogel were not significantly different at 2424 to 8352.

According to Chien and Burgi, FESI of anions in counter-EOF CZE or FESI with polarity switching is performed as follows.[40] A water plug is injected hydrodynamically, followed by electrokinetic injection at negative polarity of the sample prepared in a low conductivity matrix. The sample is normally diluted with water. During injection, the EOF pushes the water plug into the inlet vial while the anions are introduced into the capillary with high velocities due to the enhanced electric field strength in the water zone. The water plug needs to be maintained inside the capillary during electrokinetic injection because the analytes are stacked between the water plug and separation solution zone boundary inside the capillary. This boundary moves with the same velocity as the bulk EOF. The measured current during injection is monitored closely, and when this current reaches around 98% of the current with only separation electrolyte inside the capillary, the injection is stopped. This ensures that the stacking boundary is very close to the capillary inlet but is still inside the capillary. The stacked analytes are then analysed by normal counter-EOF CZE with the separation electrolyte at each end of the capillary.

Here, a simple way to perform FESI without the need to monitor the current is demonstrated in Figure 2.4.3B. For comparison, Figure 2.4.3A is the counter-EOF CZE of anions similar to that in Figure 2.4.3A except the sample diluent and separation electrolyte was 50 mM sodium phosphate at pH 7.4. It is emphasised that CZE separation in Figure 2.4.3A and 2.4.3B were the same with the separation electrolyte at both ends and an applied voltage of 20 kV.

Sample solution in Figure 2.4.3A was 100 μg/mL pravastatin (peak 1), 50 μg/mL indoprofen (peak 2) and 50 μg/mL tolfenamic acid (peak 3) prepared in separation electrolyte. Sample solution in Figure 2.4.3B was the sample in Figure 2.4.3A which was diluted 500 times with purified water. The injection in Figure 2.4.3A was 50 mbar for 5s with separation electrolyte at the capillary outlet end and in Figure 2.4.3B was -5 kV for 99s with hydrogel at the outlet. Also, a short water plug at 50 mbar for 3s was injected prior to sample injection in Figure 2.4.3B.

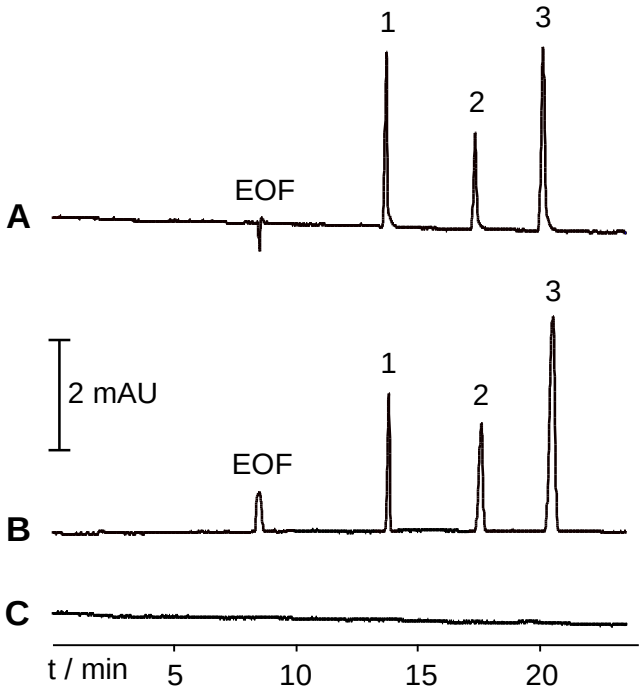

Figure 2.4.3. FESI of anionic drugs in counter-EOF CZE without manual polarity switching. (A) is a conventional hydrodynamic injection. (B) is FESI with hydrogel. (C) is FESI without hydrogel. All electropherograms were drawn in the same scale and the inserted bar indicates the absorption value. Other conditions are found in the text.

In Figure 2.4.3B, the hydrogel produced a zero net-flow and maintained the water plug and stacking boundary inside the capillary and close to the inlet end. The water plug was short and the measured current was around 98% of the current with only the separation solution. The high field strength was obtained at the tip of the capillary since only a small fraction of the capillary was filled with a low conductivity solution.[41] Comparison of the FESI injection with hydrogel (Figure 2.4.3B) with the normal injection (Figure 2.4.3A) shows that the use of the hydrogel afforded more than a 500-fold increase in peak signals. In Figure 2.4.3B, the water

plug which also served as EOF marker due to electrolyte discontinuity was also detected. The hydrogel which was at the other end of the capillary was not responsible for the positive peak. In Figure 2.4.3A, a negative peak that marked the EOF was observed due to the small amount of methanol which came from the sample stock solution. When FESI was performed without the hydrogel and the same conditions as in Figure 2.4.3B, sample introduction was not achieved and no peaks were detected, as shown in Figure 2.4.3C. It may be possible to obtain larger concentration factors but it was not attempted here.

It is emphasised that in order to maintain the water plug for at least 99 s at 5 kV injection, the net-flow must be zero which was achieved by the hydrogel. Another way to maintain the water plug is by applying a counter-pressure. We tried to apply pressure opposite to the EOF, however, the water plug was not well maintained. Another method is FESI with a longer water plug and current monitoring. The measured current during injection was monitored closely, and when this current reached around 98% of the current with only separation electrolyte inside the capillary, the injection was stopped. This ensured that the stacking boundary was very close to the capillary inlet but was still inside the capillary. The stacked analytes were then analysed by counter-EOF CZE with the separation electrolyte at each end of the capillary. This procedure was difficult to automate and lower stacking efficiency was obtained because of the smaller enhancement in field strength during injection. A short water plug is required for greater enhancement in field strength as shown in Figure 2.4.3B.

2.5 Conclusion

The use of acrylamide based hydrogel to produce zero net-flow in CZE with EOF was demonstrated. The approach is simple and can also potentially be used in electrophoretic microfluidic devices. A peak broadening effect that was possibly due to the hydrodynamic flow caused by the closure of the anodic end was directly proportional to the strength of the EOF. The strategy was successfully utilized for the fast separation of anions without significant loss in

sensitivity and separation efficiency, as well as the efficient sample stacking using electrokinetic injection under field-enhanced conditions of anions even under counter-EOF conditions. The zero net-flow in capillaries with hydrogel will be explored for other stacking systems, two-dimensional electroseparations, and electrokinetic sample microextraction/purification.

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Chapter 3

Off-line sample preparation by electrophoretic concentration using a micropipette and hydrogel

3.1 Abstract

An off-line electrophoretic sample concentration technique for charged analytes in aqueous samples is presented. As a demonstration, nine anions including inorganic ions, a dye and benzenesulfonate derivatives were enriched from a 10 mL sample solution into 20 µL electrolyte inside a glass micropipette. A hydrogel was placed at one end of the micropipette while the other end was immersed in the sample. The electric field caused the movement and concentration of anions into the high conductivity electrolyte. The technique was applied to purified, drinking and river water and was optimised by changing applied voltage and voltage application time. The MDLs after analysis by capillary electrophoresis were 1 – 19 ng/mL, 4 – 133 ng/mL and 18 – 80 ng/mL for purified, drinking and river water, respectively. The linear range was 0.002 – 0.048 to 0.1 – 2.4 µg/mL (R^2 of 0.993 – 0.999), 0.02 – 0.24 to 1.0 – 24 µg/mL (R^2 of 0.995 – 0.999) and 0.02 – 0.24 to 1.0 – 24 µg/mL (R^2 of 0.998 – 1.000), correspondingly. The intraday and interday repeatability (%RSD, n=6) was ≤ 7.4% and 14.0%, respectively. The concentration factor was from one to two orders of magnitude. The technique was directly compatible with a liquid phase analytical technique, thus eliminated the additional steps (e.g., evaporation, elution and / or reconstitution) which are typically performed in sample preparation (e.g., liquid and solid phase extraction).

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3.2 Introduction

In liquid-liquid extraction (LLE), the ideal case is to concentrate the target analytes from a large volume of sample into a much smaller volume of extraction phase.[1–3] However, for practical reasons LLE usually involves the use of an ample volume of extraction phase, with the sample and extraction phases being shaken, physically separated, and then the volume of the extraction phase is reduced by evaporation. The residue containing the extracted analytes is then usually reconstituted into a small volume of suitable solution prior to further processing.

Recently, innovative, environmentally friendly microscale techniques have emerged to reduce the amount of organic solvent used in LLE. Examples of such techniques include extraction into a solvent that is suspended in a drop (single-drop microextraction)[4–6], dispersed to increase the contact surface area (dispersive liquid-liquid microextraction)[7,8], or protected by a membrane such as a hollow fibre (hollow-fibre microextraction)[9–11]. Nevertheless, efficient extraction of polar or ionisable analytes that are more soluble in aqueous phases is still difficult. Thus, there has been increasing interest in the application of an electric field to enhance LLE of ionised and ionisable molecules from aqueous samples.[12–16] The electric field accelerates the transfer of charged molecules from the aqueous sample and then into the extraction phase.[17] In some cases, the aqueous sample and extraction phases were separated by a membrane or another liquid.[18–24] Selective enrichment of either cationic or anionic molecules can also be accomplished by manipulation of the polarity of the applied voltage. In addition, samples produced from these procedures do not normally require further processing and are often directly analysed.

In this communication, we propose an off-line electrophoretic sample concentration of charged analytes. We demonstrate the concentration of ionized analytes between two aqueous phases using an electric field, where the analytes from a low conductivity sample were concentrated to a high conductivity electrolyte. The electrolyte was immobilised inside a micropipette using a hydrogel situated at one end of the pipette. The enrichment effect was due

to known principle of the Kohlrausch adjustment of concentrations when diluted components from a sample enter the region of more concentrated electrolyte.[25–31] The hydrogel was not used to separate the sample from the electrolyte, so the proposed approach did not use any physical barrier or membrane to separate the sample and acceptor phases, nor was any organic solvent required. A range of anionic compounds, including inorganic ions, a dye, and benzenesulfonate derivatives was effectively concentrated using a simple experimental set-up. Concentration factors were from one to two orders of magnitude and the approach was applied to fortified drinking and river water.

3.3 Materials and methods

3.3.1 Reagents and stock solutions

Purified water was obtained from a Milli-Q system (Millipore, MA, USA). All reagents (acetonitrile, acrylamide, *N,N*-dimethylacrylamide, ammonium acetate, phosphoric acid, sodium hydroxide, sodium borate and potassium persulfate) were obtained from Sigma-Aldrich (New South Wales, Australia) and used as delivered. Stock electrolyte solutions of sodium phosphate pH 2.4 and 1 mol/L ammonium acetate pH 8.3 were prepared in purified water. The pH of the stock solutions was adjusted when needed using 1 mol/L sodium hydroxide. The pH and conductivity of solutions were measured using a Bench-Top Meter (Sper Scientific, Australia). All stock solutions were sonicated and filtered using 0.45 μm filter prior to use. Drinking water was collected from a tap and river water from Derwent River (New Norfolk, Tasmania, Australia).

The analytes were also obtained from Sigma-Aldrich. Analyte stock solutions of 1 mg/mL each in purified water were prepared and stored at 5-8°C when not in use. The analyte mixture consisted of potassium bromide, potassium bromate, potassium nitrate, 1,3,(6,7)-naphthalenetrisulfonic acid trisodium salt (7N), 2,6-naphthalenedisulfonic acid disodium salt

(26N), 3-hydroxynaphthalene-2,7-disulfonic acid (2N), Orange G, and 4-vinylbenzenesulfonic acid (V).

3.3.2 Hydrogel preparation

Hydrogels were prepared directly in 3 mL capacity polypropylene syringes without plunger where the narrower end was sealed with parafilm. The polymer mixture was made by mixing 55%-wt acrylamide (monomer), 99% *N,N*-dimethylacrylamide (co-monomer), 0.5 mol/L ammonium acetate at pH 8.3, purified water, and 5%-wt potassium persulfate (initiator). The ratio of monomer, co-monomer and initiator was 15:1:1.5. The mixture was heated at 60 ^{o}C for 10 min.[32]

3.3.3 Electrophoretic sample concentration

The set-up for electrophoretic concentration consisted of a high voltage power supply (Matsusada, Japan) capable of providing adjustable voltages of 0 – 30 kV (0.1 kV increments), 20 µL micropipettes with a length of 6.4 cm and an inner diameter of 0.6 mm (Microcaps, Drummond Scientific Company, USA), 3 mL disposable plastic syringes (Terumo, Philippines), and 20 mL capacity scintillation vials (Sigma-Aldrich). A hydrogel was prepared in the syringe (see *Section 3.3.2*) and the micropipette was filled with 50 mmol/L of ammonium acetate pH 8.3, which acted as electrolyte. The sample solution was stirred during electrophoretic concentration at 600 rpm.

3.3.4 Analysis of standards, samples and concentrates

Capillary electrophoresis (CE) was used to analyse the standards, samples and concentrates. CE was performed on a Beckman MDQ system (Fullerton, CA, USA). Fused-silica capillaries (60 cm long, 50 cm to the detection window) were obtained from Molex (Phoenix, AZ, USA). The separation electrolyte and voltage was 150 mmol/L sodium phosphate at pH 3 and -20 kV, respectively. The capillary was thermostated at 20°C, the detection was at 200 nm, and the sample injection was by pressure at 50 mbar for 5s.

3.4 Results and discussion

3.4.1 Scheme

The strategy is shown in Figure 3.4.1.1 (a) for anionic target analytes. The experimental set-up is shown in supporting information Figure S1. An acrylamide-based hydrogel was prepared in a cylindrical plastic tube with open ends (in this case, the tube used was a plastic syringe with the plunger removed). A micropipette was filled with an electrolyte that acted as the concentration or acceptor phase. The micropipette was partially inserted into the bottom of the hydrogel and a platinum wire was attached to the top of the hydrogel. The hydrogel prevented the extraction electrolyte from flowing out of the pipette and also supported the electrical current when voltage was applied. The other end of the micropipette and another platinum wire were then dipped into the aqueous sample. The platinum wires were connected to the voltage power supply and grounded. Voltage was applied with positive polarity at the hydrogel end, and this produced an electric field which caused the entry and electrophoretic concentration of anions from the aqueous sample to the aqueous electrolyte inside the micropipette. The net flow inside the pipette during voltage application was zero because of the hydrogel.[32] The entire electrolyte was then manually transferred and analysed using CE.

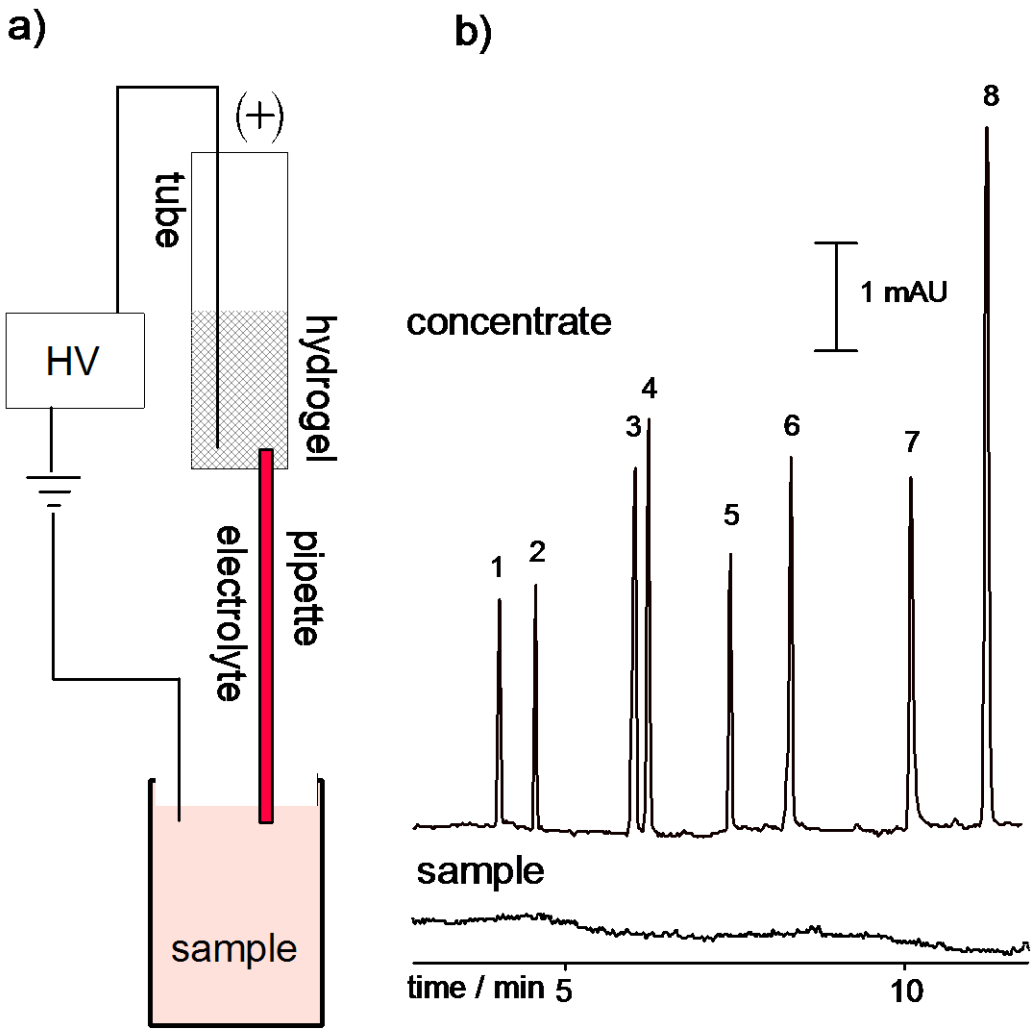

Figure 3.4.1.1. (a) Scheme for off-line electrophoretic sample concentration using a micropipette and hydrogel. (b) Electropherogram of sample (bottom) and electrolyte after sample concentration (top). For (b), the sample solution was 0.5 to 12 µg/mL of bromide (peak 1), nitrate (2), bromate (3), 7N (4), 26N (5), 2N (6), Orange G (7), V (8) in purified water. Extraction electrolyte was 50 mmol/L ammonium acetate at pH 8.3. Electrophoretic concentration was performed at 1.3 kV for 20 min. CE conditions are in the Methods Section.

3.4.2 Proof of concept

Figure 3.4.1.1 also shows an electropherogram (b) of the electrolyte (50 mmol/L ammonium acetate at pH 8.3) after electrophoretic concentration of 10 mL sample at 1.3 kV for 20 min. An electropherogram of the sample that contained three inorganic anions, one anionic dye, and four benzenesulfonate derivatives is also provided for comparison. The sample showed very small signals that were below the limit of detection (LOD) at signal to noise ratio

(S/N) of 3. However, the electrolyte analysed after application of electric field clearly showed improved detection with concentration factors from 48 to 249. The factor was calculated by dividing the CE peak area obtained from the concentrate by the corresponding area from a CE analysis of standard sample and then multiplied by the dilution factor. This translates to improvements in detection of one to two orders of magnitude.

3.4.3 Preliminary investigations on three sample matrices

The concentration technique was investigated using three water samples of different complexity and conductivity. These were purified, drinking and river water with measured conductivities of 2.7, 63.5, and 112.3 μS/cm, respectively. Using the same conditions as in *Section 3.4.2*, the voltage was varied from 0 – 3 kV. Formation of bubbles inside the micropipette was observed when the current was > 400 μA. Thus, as a system suitability test, the applied voltage was adjusted such that the observed current was 200 – 300 μA after one minute. The chosen applied voltage for purified, drinking and river water was 1.3, 0.6, and 0.4 kV, respectively. The difference in the voltages was due to the sample conductivity.

The effect of voltage application time on concentration factor was studied between 5 and 45 min. The results are shown in Figure 3.4.3.1 (a), (b), and (c) for purified, drinking and river water, respectively. The measured electrophoretic mobility using the CE conditions in Figure 3.4.3.1 (b) for bromide, nitrate, bromate, 7N, 26N, 2N, Orange G, and V was 1.0E-07, 9.0E-08, 6.8E-08, 6.6E-08, 5.5E-08, 4.9E-08, 4.0E-08, 3.6E-08 $m^2/V*s$, respectively.

In Figure 3.4.3.1 (a), the concentration factors of faster anions (bromide, nitrate, bromate, 2N, 26N, 2N) reached a maximum at 10 or 15min. The factors then decreased when the time was > 15 min, because a fraction of the concentrated anions migrated out of the micropipette. For the slower anions (Orange G and V), the maximum factors were obtained at 20 min. This was in agreement with moving boundary electrophoresis, where the concentrated zones of the high mobility ions migrated at higher velocity than the low mobility ions.

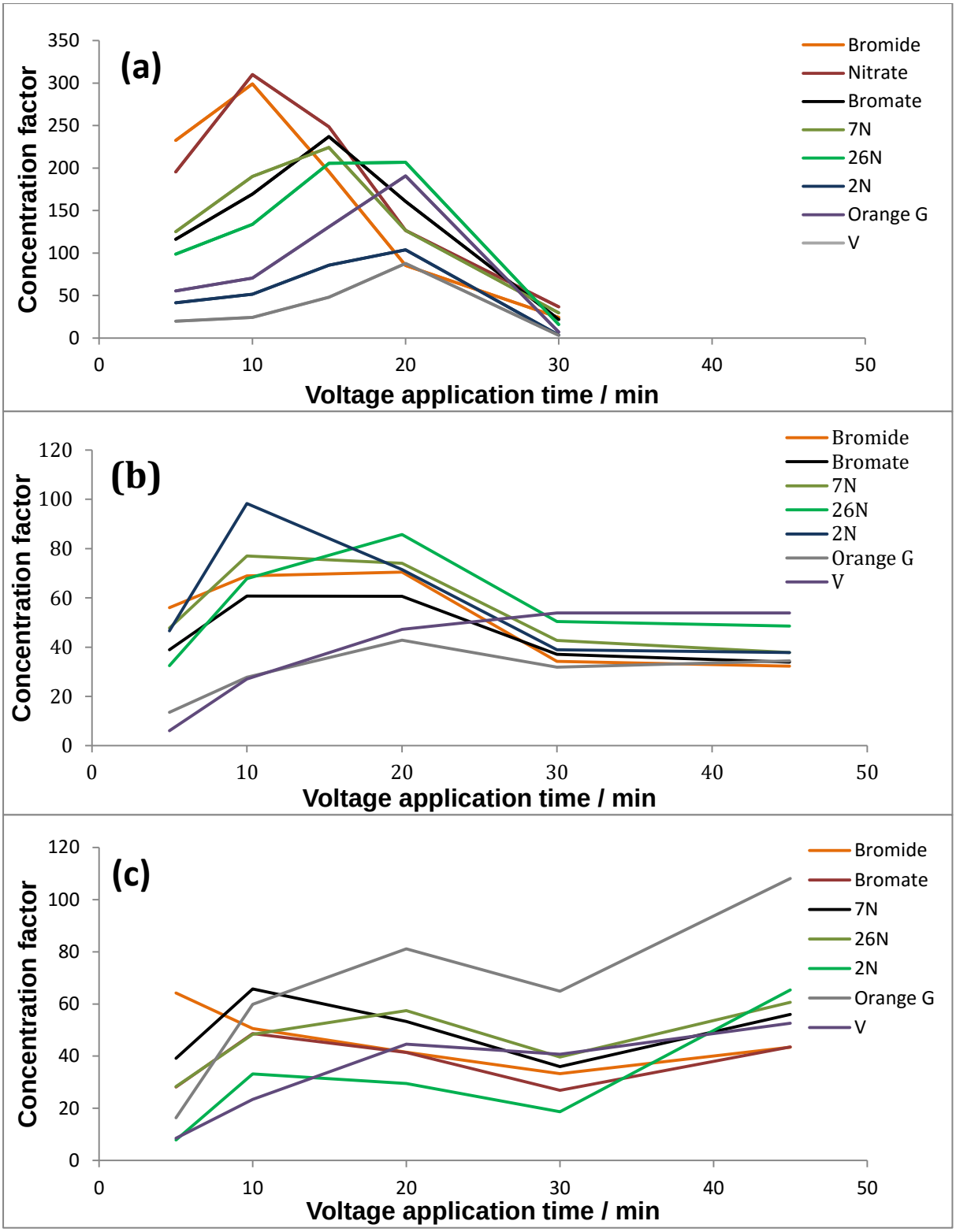

Figure 3.4.3.1. Effect of voltage application time on concentration factor for (a) purified, (b) drinking, and (c) river water. Applied voltage was 1.3, 0.6, and 0.4 kV for (a), (b), and (c), respectively. Concentration of analytes in the standard and water samples were 10 – 60 µg/mL and 0.1 – 0.6 µg/mL, respectively. The standard and sample concentrates were analysed by CE, see Method Section. The concentration factor was calculated by dividing the CE peak area obtained from the extract by the corresponding area from the CE analysis of standard sample and then multiplied by the dilution factor (=100).

The selected voltage application time for purified water was 15 min which provided good concentration factors for all analytes. For drinking water, a similar observation (see Figure 3.4.3.1 (b)) was found and the selected voltage application time was 20 min. For river water, the concentration factors behaved differently with time. This could be explained by the higher sample conductivity. The 20 min voltage application time was chosen over 45 min to reduce preparation time.

3.4.4 Analytical figures of merit

The electrophoretic concentration conditions using 50 mmol/l ammonium acetate pH 8.3 in the micropipette for purified, drinking and river water were 1.3 kV and 15 min, 0.6 kV and 20 min, and 0.4 kV and 20 min, respectively. The analytical figures of merit for purified, drinking and river water are shown Table 3.4.4.1 (a), (b), and (c), respectively. The method detection limit (MDL) and method quantitation limit (MQL) were obtained at S/N of 3 and 10, respectively. The analyte concentrations in the sample for both the MDL and MQL include the off-line sample concentration by EC and CZE-UV analysis. Chloride and nitrate were found in drinking and river water samples after concentration, thus, the applicable analytical values were not provided. Supporting information Figure S2 shows representative blank electropherograms of unfortified drinking and river water samples that were subjected to the procedure.

The linear range was assessed in duplicate for five analyte concentrations by adding aliquots of standard to the water matrix. The sample preparation technique was linear for at least an order of magnitude. The correlation coefficients R^2 were 0.993 – 1.000. The MDL and MQL values were lowest for purified water (MDL = 0.001 – 0.019 μg/mL, MQL = 0.003 – 0.062 μg/mL). MDL and MQL for drinking (MDL = 0.019 – 0.133 μg/mL, MQL = 0.063 – 0.444 μg/mL) and river (MDL = 0.018 – 0.080 μg/mL, MQL = 0.061 – 0.267 μg/mL) water were similar. Indeed, the concentration factors were better for purified (48 – 249) compared to drinking (43 – 86) and river (30 – 81) water. These results suggest that the present system is more effective

for low conductivity samples. The decrease in the concentration factor was a consequence of the sample matrix which caused a change in the analyte's electrophoretic mobility. For instance, a decrease in mobility will also decrease the CFs for a constant voltage application time. However, the change in mobility by the change in sample matrix is unpredictable and analyte-depending. The %recovery values were also better for purified (9.6 – 49.7%) compared to drinking (8.6 – 17.1%) and river (5.9 – 16.2%) water. The %recovery was calculated by dividing the obtained concentration factor with the theoretical concentration factor for complete analyte concentration (volume of sample / volume of extraction electrolyte = 500) and given in percentages. The values are low because the concentration technique is non-exhaustive. For repeatability (%RSD, n=6), the analyte concentrations in purified water were 0.05 – 1.2 μg/mL and in drinking and river water were 0.5 – 12 μg/mL. Supporting information Figure S2 also shows representative electropherograms of fortified drinking and river water samples that were subjected to the procedure. The values for intraday and interday repeatability were 1.4 – 7.4% and 2.3 – 14.0%, respectively.

Table 3.4.4.1. Analytical figures of merit, concentration factors and recovery obtained for different water samples

(a)	Linear range (µg/mL)	Regression (y=)	R^2	MDL[a] (µg/mL)	MQL[b] (µg/mL)	Repeatability, intraday, (%RSD, n=6)	Repeatability, interday, (%RSD, n=6)	Concentration factor[c]	Recovery[d] (%)
Bromide	0.004 - 0.2	1.69E+05x + 2.2	0.999	0.002	0.006	4.0%	4.5%	196	39.2%
Nitrate	0.002 - 0.1	2.70E+05x + 62	0.993	0.001	0.003	7.4%	7.2%	249	49.7%
Bromate	0.048 - 2.4	2.32E+05x + 38	0.993	0.019	0.062	3.8%	4.6%	237	47.4%
7N	0.024 - 1.2	5.87E+05x + 122	0.999	0.009	0.030	3.2%	4.9%	224	44.9%
26N	0.008 - 0.4	3.25E+05x + 141	0.997	0.003	0.010	7.4%	8.7%	206	41.1%
2N	0.024 - 1.2	5.65E+5x + 220	0.996	0.010	0.033	6.3%	9.9%	86	17.1%
Orange G	0.016 - 0.8	1.44E+05x + 55	0.995	0.007	0.023	4.6%	11.1%	131	26.1%
V	0.010 - 0.5	8.78E+04x + 12	0.998	0.002	0.007	5.8%	14.0%	48	9.6%

(b)	Linear range (µg/mL)	Regression (y=)	R^2	MDL[a] (µg/mL)	MQL[b] (µg/mL)	Repeatability, intraday, (%RSD, n=6)	Repeatability, interday, (%RSD, n=6)	Concentration factor[c]	Recovery[d] (%)
Bromide	0.040 - 4.0	2.03E+5x + 110	0.996	0.019	0.063	4.6%	3.2%	70	14.1%
Bromate	0.480 - 24.0	2.92E+5x + 153	0.996	0.133	0.444	1.4%	2.3%	61	12.1%
7N	0.240 - 12.0	2.75E+5x + 124	0.996	0.095	0.316	1.6%	2.4%	74	14.8%
26N	0.080 - 4.0	1.35E+5x + 59	0.997	0.035	0.118	2.0%	2.8%	86	17.1%
2N	0.240 - 12.0	2.26E+5x + 76	0.998	0.064	0.214	4.2%	3.8%	72	14.3%
Orange G	0.160 - 8.0	2.47E+5x - 24	0.996	0.056	0.188	3.9%	5.0%	43	8.6%
V	0.100 - 5.0	4.27E+5x - 14	0.999	0.022	0.072	3.8%	5.7%	47	9.4%

Table 3.4.4.1 continued. Analytical figures of merit, concentration factors and recovery obtained for different water samples

(c)	Linear range (µg/mL)	Regression (y=)	R^2	MDL[a] (µg/mL)	MQL[b] (µg/mL)	Repeatability, intraday, (%RSD, n=6)	Repeatability, interday, (%RSD, n=6)	Concentration factor[c]	Recovery[d] (%)
Bromide	0.040 - 4.0	1.43E+5x + 67	1.000	0.018	0.061	3.0%	5.1%	42	8.3%
Bromate	0.480 - 24.0	1.60E+5x + 61	1.000	0.080	0.267	3.4%	7.3%	53	10.7%
7N	0.240 - 12.0	1.72E+5x + 72	0.999	0.068	0.226	2.7%	4.8%	57	11.5%
26N	0.080 - 4.0	7.82E+4x + 46	1.000	0.029	0.095	2.1%	4.5%	30	5.9%
2N	0.240 - 12.0	1.19E+5x + 100	0.998	0.065	0.218	6.2%	4.5%	42	8.5%
Orange G	0.160 - 8.0	1.38E+4x + 29	1.000	0.051	0.168	4.7%	7.2%	81	16.2%
V	0.100 - 5.0	2.25E+5x + 76	0.999	0.027	0.091	3.5%	7.7%	45	8.9%

[a] MDL was calculated based on a S/N = 3

[b] MQL was calculated based on a S/N = 10

[c] The concentration factor was calculated by dividing the CE peak area obtained from the extract by the corresponding area from a CE analysis of standard sample and then multiplied by the dilution factor. The electrophoretic concentration conditions were 1.3 kV and 15 min (a), 0.6 kV and 20 min (b), and 0.4 kV and 20 min (c).

[d] The %recovery was calculated by dividing the obtained concentration factor with the theoretical concentration factor for complete analyte concentration (volume of sample / volume of extraction electrolyte = 500) and given in percentages.

3.5 Conclusion

A green microscale method to concentrate charged analytes in dilute aqueous samples into an electrolyte inside a micropipette using an electric field was presented. The immobilisation of the electrolyte by plugging one end of the pipette with an electrically conductive hydrogel was key to the success of the method. Electrophoretic concentration of purified, drinking and river water at 0.4 – 1.3 kV for 15 – 20 min provided analyte concentration factors of 42 to 249. The analyte MDLs were from 0.001 – 0.133 μg/mL after CE analysis. The use of sensitive analytical techniques would further improve the MDLs. The approach is simple and could be easily adopted in other laboratories.

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3.7 Supporting information

Figure S1

- The hydrogel was prepared in an open ended tube. A platinum wire was attached to the hydrogel.
- An electrolyte filled micropipette (20 µL) was attached to the hydrogel and immersed into the sample solution
- The intense yellow colour in the electrolyte was due to the enrichment of the coloured dye Orange G
- Dilute aqueous sample solution containing anionic ions and coloured dye
- A platinum wire was dipped in the sample solution

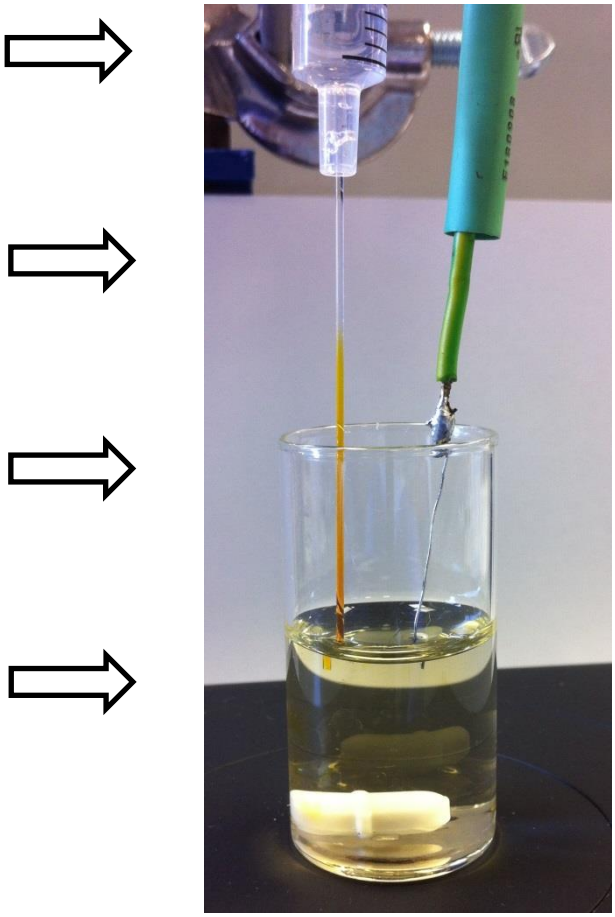

Figure S1. Experimental set-up for off-line electrophoretic concentration of charged analytes.

Figure S2

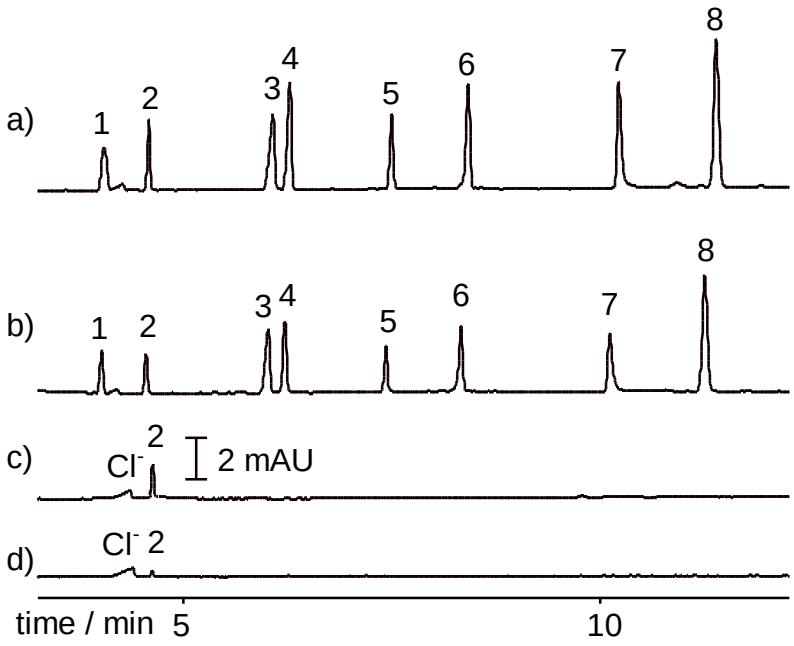

Figure S2. Electrophoretic concentration of river ((a) and (c)) and drinking ((b) and (d)) water. Electropherograms obtained after CE analysis of concentrate from spiked ((a) and (b)) and blank ((c) and (d)) water samples. Peak identification is the same as for Figure 3.4.1.1 (b).

Chapter 4

Electrophoretic concentration and sweeping-micellar electrokinetic chromatography analysis of cationic drugs in water samples

4.1 Abstract

Sample preparation by electrophoretic concentration, followed by analysis using sweeping-micellar electrokinetic chromatography, was studied as a green and simple analytical strategy for the trace analysis of cationic drugs in water samples. Electrophoretic concentration was conducted using 50 mmol/L ammonium acetate at pH 5 as acceptor electrolyte. Electrophoretic concentration was performed at 1.0 kV for 45 min and 0.5 kV and 15 min for purified and 10-fold diluted wastewater samples, respectively. Sweeping-micellar electrokinetic chromatography was with 100 mmol/L sodium phosphate at pH 2, 100 mmol/L sodium dodecyl sulfate and 27.5%-v/v acetonitrile as separation electrolyte. The separation voltage was -20 kV, UV-detection was at 200 nm, and the acidified concentrate was injected for 36 s at 1 bar (or 72% of the total capillary length, 60 cm). Both purified water and 10-fold diluted wastewater exhibited a linear range of two orders of concentration magnitude. The coefficient of determination, and intra- and interday repeatability were 0.991-0.997, 2.5-6.2 and 4.4-9.7 %RSD (n=6), respectively, for purified water. The values were 0.991-0.997, and 3.4-7.1 and 8.7-9.8 %RSD (n=6), correspondingly, for 10-fold diluted wastewater. The method detection limit was in the range from 0.04-0.09 ng/mL and 1.20-6.97 ng/ml for purified and undiluted waste water, respectively.

*All of this research contained in this chapter has been published as A. Wuethrich, P.R. Haddad, J.P. Quirino, Electrophoretic concentration and sweeping-micellar electrokinetic chromatography analysis of cationic drugs in water samples, *J. Chromatogr. A.* 1401, 84–88, **2015**.

4.2 Introduction

Electric field-assisted sample preparation has attracted much recent interest because of the enhanced analyte extraction selectivity resulting from the introduction of an electric field. The goals are to achieve efficient sample clean-up within a short period of time, at low cost, and in an environmentally-responsible way. A useful strategy to accomplish these goals is to superimpose an electric field onto traditional extraction techniques, such as liquid-liquid extraction (LLE), solid-phase extraction (SPE), and dialysis. The electric field enhances the transfer of charged analytes across a physical boundary, which can be the interface of two immiscible liquids (i.e., electroextraction)[1], a solid-liquid phase (i.e., electric field-assisted solid-phase extraction, EA-SPE)[2–5] or two or more miscible phases separated by a membrane or filter (i.e., electromembrane extraction or EME[6,7] and electrodialysis[8]). In EA-SPE, the electric field can also be used to support the elution of the analyte from the sorbent. High enrichment factors and low limits of detection have been achieved by the implementation of electroextraction, EME, and EA-SPE.[9,10]

Concentration of microliter scale sample volumes using an electric field and careful manipulation of sample and supporting electrolyte was proposed more than two decades ago.[11–13] We have previously reported a selective electrophoretic concentration (EC) scheme for ionised and ionisable analytes from aqueous or water samples. This scheme was initially demonstrated for ionised anionic analytes. Eight anionic analytes were injected electrokinetically from 10 mL of a low conductivity sample into 20 μL of acceptor electrolyte held inside a micropipette.[14] The principle of concentration is based on field-enhanced sample injection where the analytes from a low conductivity sample were injected into a high conductivity electrolyte (i.e., acceptor electrolyte) inside the micropipette.[15] EC did not use organic solvents or a physical barrier to separate the sample and acceptor phases. Using only an electric field as driving force, the concentrations of anionic analytes in the sample were increased by up to more than two orders of concentration. The experimental set-up for EC can

be found in Figure 3.4.1.1 (a) of Chapter 3. Briefly, a micropipette filled with acceptor electrolyte was inserted into to a plug of hydrogel housed in a syringe and the other end of the micropipette was submerged into the sample solution. The hydrogel at the top end of the micropipette prevented the flow of electrolyte out of the pipette due to gravity. During voltage application, the hydrogel also supported the electric current and provided a zero net flow of liquid inside the pipette.[16] The anions were concentrated in the acceptor electrolyte as a result of electrophoretic migration towards the anode situated at the hydrogel end of the pipette.

Popular approaches to improve the detection sensitivity of CE techniques (including micellar electrokinetic chromatography (MEKC)) coupled to UV detection are based on on-line sample concentration or stacking.[17–19] Sweeping, a mode of on-line sample concentration in MEKC, uses a pseudostationary phase (in this case, micelles) to accumulate the analytes into sharp zones.[20,21] Concentration factors (CF) of one to more than three orders of magnitude can be achieved.[22,23] However, sample preparation is often required in order to convert the sample into a form which is amenable to sweeping-MEKC, in particular by the use of a diluent devoid of the micelles. Examples of such preliminary sample treatment procedures include solvent-solvent extraction[24], dispersive liquid-liquid microextraction[25,26], magnetic solid particle extraction[27], solid-phase microextraction[28], and single drop microextraction[29]. In these techniques, the sample was ultimately extracted into an organic solvent, requiring that the extracts were first evaporated and the target analytes then reconstituted in a micelle-free diluent prior to sweeping-MEKC.

In the present study, EC was examined for the off-line sample preparation of cationic drugs in simple and complex water samples. Promethazine, dibucaine, doxepin, verapamil, and alprenolol were used as model ionisable analytes, while purified and waste water were used as sample matrices. EC provided a micelle-free concentrate, thus a sweeping-MEKC method was also optimised to separate the target analytes and to obtain good analyte detection sensitivities. In EC, the type and concentration of the acceptor electrolyte and the voltage application time

were investigated. In sweeping-MEKC, the injection time and the effect of acidic buffer addition to the concentrate were studied. The performance of the combined analytical procedure of EC and sweeping-MEKC (i.e., linearity, method detection limit (MDL), method quantitation limit (MQL), repeatability, and concentration factor (CF)) was also investigated. MDL and MQL are the minimum analyte concentrations in the sample for detection and quantification of the whole method (i.e., EC combined with sweeping-MEKC).

4.3 Materials and methods

4.3.1 Reagents and stock solutions

Purified water was obtained from a Milli-Q system (Millipore, MA, USA). All reagents (acetonitrile, acrylamide, ammonium acetate, phosphoric acid, sodium hydrogen carbonate, sodium carbonate, 2-amino-2-hydroxymethyl-propane-1,3-diosodium hydroxide (Tris), and sodium dodecyl sulfate (SDS)) were obtained from Sigma-Aldrich (New South Wales, Australia) and used as delivered. Stock electrolyte solutions of sodium phosphate at pH 2 and 0.5 mol/L ammonium acetate at pH 5 were prepared in purified water. The pH of the stock solutions was adjusted when needed using 1 mol/L sodium hydroxide or acetic acid. The pH and conductivity of solutions were measured using a Bench-Top Meter (Sper Scientific, Australia). All stock solutions were sonicated and filtered using 0.45 μm filter prior to use. Wastewater effluent was donated from a local sewerage company (TasWater, Moonah, Australia) and filtered through a paper filter (Grade 1, Whatman, GB) prior to use. The analytes were also obtained from Sigma-Aldrich. Analyte stock solutions of 1 mg/mL each in methanol were prepared and stored at 5-8°C when not in use. The analyte mixture consisted of hydrochloride salts of promethazine, dibucaine, doxepin, verapamil, alprenolol, and clomipramine (internal standard).

4.3.2 Hydrogel preparation

Hydrogels were prepared directly in 3 mL capacity polypropylene syringes without plunger where the narrower end was sealed with parafilm. The polymer mixture was made by mixing 700 µL of 50%-wt aqueous acrylamide (monomer), 120 µL of 0.5 mol/L ammonium acetate at pH 5, 320 µL purified water, and 60 µL of 5%-wt potassium persulfate (initiator). The mixture was heated at 60 ˚C for 10 min.

4.3.3 Electrophoretic sample concentration

The set-up for EC consisted of a high voltage power supply (Matsusada, Japan) capable of providing voltages up to 30 kV (0.1 kV increments), two platinum electrodes connected to the voltage power supply, 20 µL micropipettes with a length of 6.4 cm and an inner diameter of 0.6 mm (Microcaps, Drummond Scientific Company, USA), 3 mL disposable plastic syringes (Terumo, Philippines), and 50 mL capacity polypropylene sample vials (Sarstedt, Australia). The sample volume in all experiments was 20 mL. Magnetic stirring of the sample (LabCo, SA, Australia) was performed with a stirrer bar (length x width = 3 x 1 mm).

EC was performed as in Chapter 3 except that the cations were concentrated with the cathode at the hydrogel end of the pipette. When the current was >600 µA, bubble formation inside the micropipette was observed. The voltage which provided a current of <500 uA after 4 min was selected for each sample. After EC, a 15 µL aliquot of the concentrate and 0 to 3 µL of 1 mol/L sodium phosphate at pH 2 were transferred into the vial for analysis.

4.3.4 CE procedure

The standards, samples and concentrates were analysed on a G1600 Agilent 3D CE system (Waldbronn, Germany). Fused-silica capillaries (60 cm total length, 51.5 cm to the detection window) were obtained from Molex (Phoenix, AZ, USA). The separation of the model analytes was achieved using a background solution of 100 mmol/L sodium phosphate at pH 2 containing 100 mmol/L SDS and 27.5% acetonitrile. The separation voltage was -20 kV. The

capillary was thermostated at 20°C and detection was by UV-absorption at 200 nm. Injection of the sample was at 50 mbar for 3 or 60 s and 1 bar (flush mode) for 36 s, respectively.

4.4 Results and discussion

4.4.1 Selection of the acceptor electrolyte

The type and concentration of the acceptor electrolyte were selected using purified water as sample matrix. Different types/pHs of buffers were first tested and then the effect of buffer concentration of the chosen buffer was studied. The sample solution was 20 mL purified water spiked with 100 ng/mL of the analyte mixture. EC was performed at 1 kV for 5 to 60 min. The concentrates (without acid addition) were analysed by sweeping-MEKC using an injection of 50 mbar for 60 s, which allowed adequate detection of the analytes in the concentrate. The CF due to EC (CF_{EC}) was calculated by dividing the corrected peak area of the analytes by the corrected peak area of the standards and then multiplied by the dilution factor.

In EC, the acceptor electrolyte conductivity must be higher than that of the sample. The conductivity of the purified water fortified with analytes was 0.06 mS/cm. The buffers studied were Tris (68 mmol/L, pH 7, 4.7 mS/cm), sodium phosphate (50 mmol/L, pH 2, 4.4 mS/cm), sodium hydrogen carbonate/sodium carbonate (25 mmol/L, pH 11, 4.7 mS/cm), ammonium acetate (50 mmol/L, pH 5, 4.7 mS/cm), and ammonium acetate (50 mmol/L, pH 9, 4.7 mS/cm). The conductivities of the tested buffers were similar in order to provide similar electric field strengths during EC. The highest CF_{EC} was obtained with 50 mmol/L ammonium acetate at pH 5 with EC at 1 kV for 50 min. The concentration of ammonium acetate buffer at pH 5 was then varied at 25, 50, 75 and 100 mmol/L. The highest CF_{EC} was also obtained with 50 mmol/L ammonium acetate at pH 5 for 50 min.

Figure 4.4.1.1 shows the dependence of the CF_{EC} on the voltage application time for the selected electrolyte (50 mmol/L ammonium acetate at pH 5). The CF_{EC} of all five cationic drugs

increased steadily from 11-26 to 97-243 from 5 to 50 min. At 55 min, the cationic drugs started to migrate out the micropipette, causing the CF_{EC} to decrease to 57-102. It is noted that the analyte migration into the hydrogel was due to electrophoretic migration and not by fluid flow caused by electroosmosis. Closing one end of the pipette by the use of a hydrogel cancelled the electroosmotic flow directed to the cathode. The observed current with sample solution inside the pipette and sample solution was below 10 μA due to the low conductivity of the sample solution. The measured current from the start to the end of EC was, however, in the range from 90 to 180 μA. This clearly indicated that the sample solution did not enter the micropipette and the migration of the analytes was due only to electrophoretic mobility.

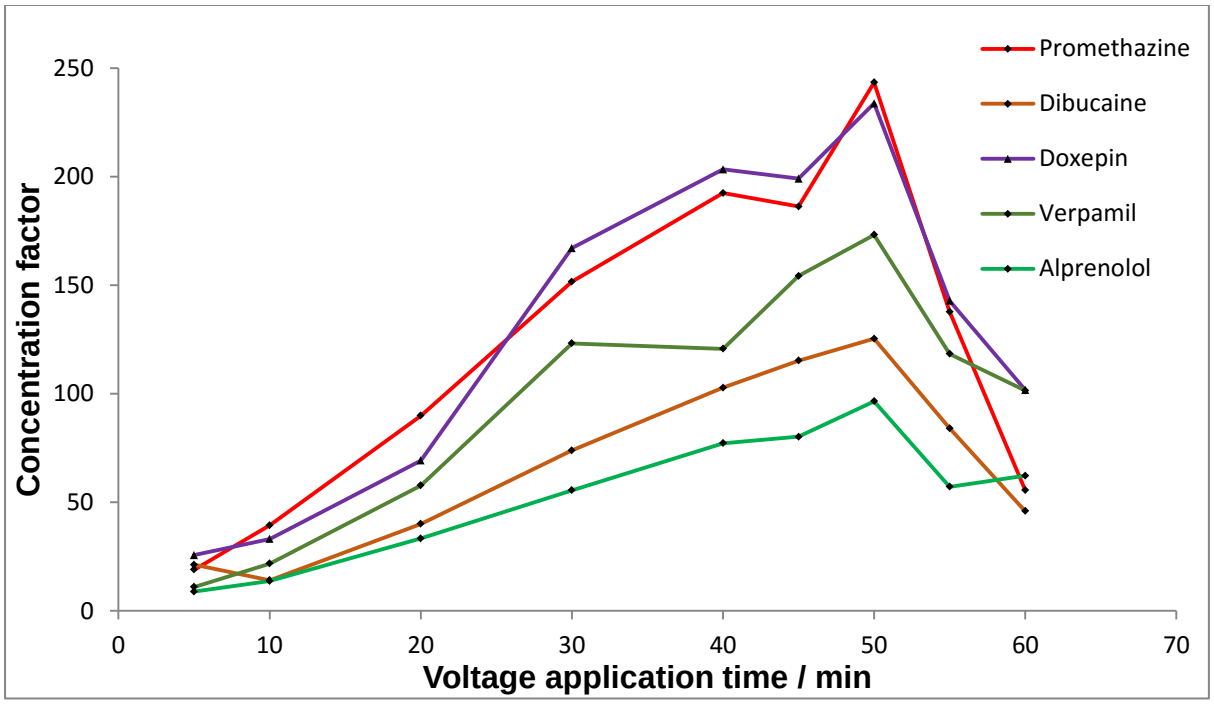

Figure 4.4.1.1. Effect of voltage application time on concentration factor of cationic drugs in purified water. Applied voltage was 1.0 kV. The analyte concentration in the sample was 100 ng/mL. The concentration factor was calculated by dividing the CE peak area obtained from the concentrate by the peak area from a standard sample and then multiplied by the dilution factor (=500). CE analysis of standard and sample see Section 4.3.

Also in Figure 4.4.1.1, the CF_{EC} is affected by the electrophoretic mobility of the analytes. The CF_{EC} at 5 to 50 min for the higher mobility cations promethazine and doxepin were larger than the other drugs. This is reminiscent of the bias in electrokinetic injection in CE where there is preference for the injection of high mobility analytes. The electrophoretic mobilities of promethazine, doxepin, verapamil, dibucaine and alpenolol were 2.06E-06, 2.03E-06, 1.99E-06, 1.79E-06, 1.73E-06 $m^2/V*s$, respectively. The mobility was determined by CZE using 50 mmol/L ammonium acetate at pH 5 as separation electrolyte.

4.4.2 Optimisation of sweeping

In order to increase the sensitivity of the sweeping-MEKC method, the sample injection length was studied. Hydrodynamic injections of 100 ng/mL analyte mixture prepared in the optimised acceptor electrolyte were performed at 1 bar for 12 to 42 s (145 to 507 mm injection length). The sensitivity enhancement factor of sweeping (SEF_{sweep}) was calculated by dividing the corrected peak area of 1 µg/mL analyte standard obtained by sweeping-MEKC with that of a 50 µg/mL analyte standard injected for 3 s at 50 mbar. The injection of 36 s at 1 bar provided high SEF_{sweep} values (see Table 4.4.3) without compromise to resolution. Figure 4.4.2.1 shows typical electrochromatograms obtained from sample injections of the 3 s at 50 mbar (a) and 36 s at 1 bar (b). The injection regimen in (b) was then used to analyse the EC concentrates.

The analysis of the EC concentrates by sweeping-MEKC showed poor resolution and peak shapes compared to the analysis of the standard mixture prepared in the acceptor electrolyte. This was attributed to the difference in the composition between the EC concentrate and the standards. The addition of concentrated (1 mol/L) sodium phosphate at pH 2 into the EC concentrate was then investigated to improve the performance of sweeping-MEKC analysis of the EC concentrate. The addition of this solution decreased the pH and increased the conductivity of the concentrate. 1, 2, and 3 µL of 1 mol/L sodium phosphate was added to a 15 µL aliquot of the concentrate. The addition of 2 and 3 µL of sodium phosphate caused significant sharpening and baseline resolution of all five peaks. For further studies, 2 µL of 1

mol/L sodium phosphate was added into 15 µL of the EC concentrate prior to sweeping-MEKC with injection for 36 s at 1 bar.

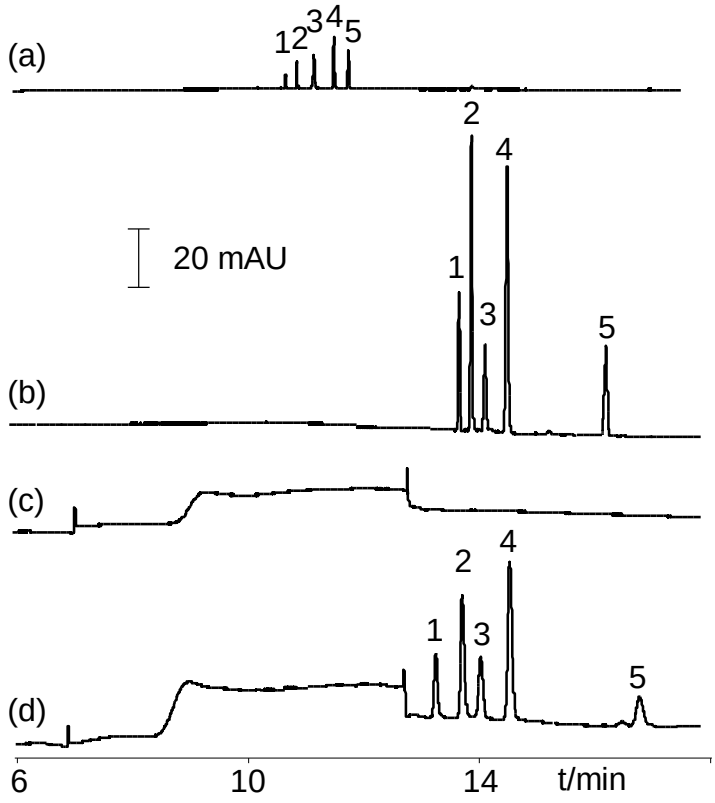

Figure 4.4.2.1. Effect of sample injection regimen on sweeping-MEKC of cationic drugs. Electrochromatograms obtained from (a, b) analyte standards and acidified EC concentrates of 1:9 dilution of wastewater in purified water (c) and spiked with analyte mixture (d). Analyte concentration and injection for the standards and sample solutions were (a) 50 µg/mL and 3s at 50 mbar, (b) 1 µg/mL and 36 s at 1 bar, and (d) 100 ng/mL and 36 s at 1 bar, respectively. Voltage and voltage application time of electrophoretic concentration in (c, d) was 0.5 kV and 15 min, respectively. Peak identities; (1) promethazine, (2) dibucaine, (3) doxepin, (4) verapamil, and (5) alprenolol. Other conditions can be found in Section 4.3.4 and 4.4.4.

4.4.3 Analytical figures of merit for cationic drugs spiked in purified water

Table 4.4.3(a) shows the analytical performance of EC and sweeping-MEKC under optimised conditions. The MDL and MQL were calculated at a signal-to-noise ratio (S/N) of 3 and 10, respectively. The MDL and MQL for all five analytes were in the range 0.04-0.09 and 0.1-0.3 ng/mL, respectively. The linear range was determined from sample solutions with 1, 5, 10, 20, 50, and 100x the MQL analyte concentrations (in duplicate). Good coefficients of determination (R^2) of 0.991 to 0.997 were obtained for calibration plots. The repeatability was determined using a sample with a concentration of 50x MQL of each analyte. Clomipramine at a concentration of 10 ng/mL was used as an internal standard. The percentage relative standard deviation (%RSD, n=6) values for intra- and interday were 2.5-6.2% and 4.4-9.7%, respectively. The total or overall concentration factor (CF_{total}) was calculated from the quotient of the corrected peak area from the EC concentrate obtained by the sweeping-MEKC method and the corrected peak area from a 50 µg/mL standard solution analysed under typical MEKC conditions (injection for 3 s at 50 mbar). High CF_{total} values of more than four orders of magnitude were obtained for all test analytes by the combination of EC with sweeping-MEKC.

Table 4.4.3 (a) provides the values for CF_{total}, SEF_{sweep}, and CF_{EC}. The SEF_{sweep} was approximated by CF_{total}/CF_{EC}, where CF_{EC} were the values from Figure 4.4.1.1 at 50 min voltage application time. From Table 4.4.3 (a), it is shown that the individual contributions of sweeping (SEF_{sweep}) and EC (CF_{EC}) to the overall CF (CF_{total}) were each approximately two orders of magnitude.

Table 4.4.3. Analytical figures of merit and concentration factors obtained for (a) purified water and (b) 1:9 diluted wastewater effluent.

(a)	Linear range (ng/mL)	Regression (y=)	R^2	MDL[a] (ng/mL)	MQL[b] (ng/mL)	Repeatability, intraday, (%RSD, n=6)	Repeatability, interday, (%RSD, n=6)	CF_{total}[c]	SEF_{sweep}[d]	CF_{EC}[e]
Promethazine	0.20-20	2.204x-1.806	0.997	0.06	0.20	6.2	9.7	44991	185	243
Dibucaine	0.13-13	1.469x-5.724	0.995	0.04	0.13	5.5	7.6	40200	322	125
Doxepin	0.23-23	0.623x-2.000	0.992	0.07	0.23	2.5	4.4	17415	74	234
Verpamil	0.30-30	0.623x-1.733	0.991	0.09	0.30	3.0	6.5	23556	136	173
Alprenolol	0.30-30	0.933x-1.541	0.996	0.09	0.30	4.7	7.3	10387	107	97

(b)	Linear range (ng/mL)	Regression (y=)	R^2	MDL[a] (ng/mL)	MQL[b] (ng/mL)	Repeatability, intraday, (%RSD, n=6)	Repeatability, interday, (%RSD, n=6)	CF_{total}[c]	SEF_{sweep}[d]	CF_{EC}[e]
Promethazine	0.63-63	0.919x-0.427	0.997	0.19	0.63	4.3	9.3	3534	185	19
Dibucaine	0.40-40	0.061x-0.068	0.991	0.12	0.40	7.1	9.8	5121	322	16
Doxepin	1.00-100	0.308x-0.250	0.995	0.30	1.00	6.6	9.5	2193	74	30
Verpamil	0.57-57	0.077x-0.212	0.996	0.17	0.57	6.4	9.8	6444	136	47
Alprenolol	2.33-233	0.897x-0.804	0.997	0.70	2.33	3.4	8.7	1297	107	12

[a] MDL was calculated based on a S/N = 3

[b] MQL was calculated based on a S/N = 10

[c] The total concentration factor (CF_{total}) was calculated by dividing the corrected MEKC peak area obtained from the concentrate by the corresponding area from a 50 µg/mL standard injected for 3 s at 50 mbar and then multiplied by the dilution factor. The electrophoretic concentration conditions were 1.0 kV and 50 min (a) and 0.5 kV and 15 min (b) using 50 mmol/L ammonium acetate at pH 5 as acceptor electrolyte.

[d] The SEF_{sweep} was calculated from CF_{total}/CF_{EC}

[e] The CF_{EC} was calculated as in Figure 4.6.1 (50 min).

4.4.4 Analytical figures of merit for the cationic drugs spiked in wastewater effluent

The wastewater sample had a conductivity of 2.3 mS/cm, thus before EC dilution of the sample with purified water was required to lower the conductivity. The effect of voltage application time on the CF_{total} was studied using dilutions of 1:2, 1:4, 1:9, 1:19 (v:v) with purified water. The diluted wastewater was spiked with the analyte standards (the final concentration was 100 ng/mL of each drug). Using 50 mmol/L ammonium acetate at pH 5 as the acceptor electrolyte, EC was performed at 0.5 kV for 15, 30, and 45 min. After analysis of the EC concentrate by sweeping-MEKC, the CF_{total} was calculated based on the diluted sample. The values were 196-494, 386-891, 830-2936 and 554-2380 for the 1:2, 1:4, 1:9 and 1:19 dilutions, respectively. However, when the dilution factor was considered, the 1:9 dilution provided the highest CF_{total}. The CF_{total} started to level off at 1:9 to the 1:19 dilution, as it is shown by the similar values for the 1:9 and 1:19 dilutions. This suggests a non-linear dependence of the CF on the sample conductivity. A much higher dilution of the wastewater might increase CFs, but would not be reasonable. The 1:9 dilution was used to study the voltage application time in more detail from 5 to 60 min. An increase of the CF_{total} was observed from 5 to 15 min to values of 283-1516 to 1409-6524. There was no significant change in the CF_{total} from 15 to 60 min. Figure 4.4.2.1 shows representative electrochromatograms obtained from the optimum EC (0.5 kV for 15 min) of the unspiked (c) and spiked (d) waste water sample.

Table 4.4.3 (b) shows the analytical figures of merit, repeatability, and concentration factors of EC and sweeping-MEKC determined from a 1:9 dilution of wastewater under optimised conditions. CF_{total} and CF_{EC} were calculated as in Section 4.4.3. The SEF_{sweep} was assumed the same values as in (a) in order to approximate the CF_{EC} by CF_{total}/SEF_{sweep}. The CF_{EC} was approximately one order of magnitude lower than the CF_{EC} for the same analyte in purified water. This was attributed to the higher conductivity of diluted wastewater (224 μS/cm). The MDL and MQL were 0.12-0.70 and 0.40-2.33 ng/mL, respectively. Analyte concentrations of 1, 5, 10, 20, 50, and 100x the MQL (in duplicate) were used to determine the linearity. All five

cationic drugs provided good R^2 of 0.991 to 0.997. The repeatability was determined using a sample spiked to 50x MQL concentration of each analyte. 20 ng/mL clomipramine was added to the sample and served as an internal standard. The %RSD (n=6) values for intra- and interday were 3.4-7.1% and 8.7-9.8%, respectively.

The true values of MDL and MQL for the wastewater samples can be approximated by considering the dilution factor of 10. These values were 1.2-7.0 and 4.0-23.3 ng/mL, respectively. The concentration factor of EC for an undiluted wastewater sample was then from 1-3, because of the dilution. In effect, the EC therefore served primarily as a simple, fast (15 min), and green (no organic solvents used) sample clean-up procedure for the tested wastewater sample. In addition, the concentrate was analysed by sweeping-MEKC without complicated steps. By contrast, in the sample preparation of similar drugs[24] prior to sweeping-MEKC analysis, a longer and tedious LLE was performed using dichloromethane. The wastewater sample was made alkaline, extracted three times with the organic solvent, centrifuged, dried under vacuum and finally reconstituted by sonication in the separation electrolyte.

4.5 Conclusion

The new off-line sample preparation technique of electrophoretic concentration or EC was demonstrated and optimised for lower mobility and positively ionisable drugs. EC was applied to a complex sample matrix (i.e., waste water effluent) and combined with stacking CE methodology (i.e., sweeping-MEKC) for the first time. EC of model cationic drugs from purified water and 10-times diluted wastewater samples afforded concentration factors or CF_{EC} from 12-243. Sweeping-MEKC provided sensitivity enhancement factors or SEF_{sweep} of 74-322 on the acidified EC-concentrate. Sweeping-MEKC analysis of the EC concentrate from purified water afforded very low MDLs of 40-90 pg/mL. In the case of a wastewater sample, dilution was necessary for electrophoretic concentration and thus MDLs were higher at 1.2-7.0 ng/mL. The

combination of EC with sweeping-MEKC provided a sensitive, simple, fast and environmentally-friendly analytical strategy for trace analysis of water samples.

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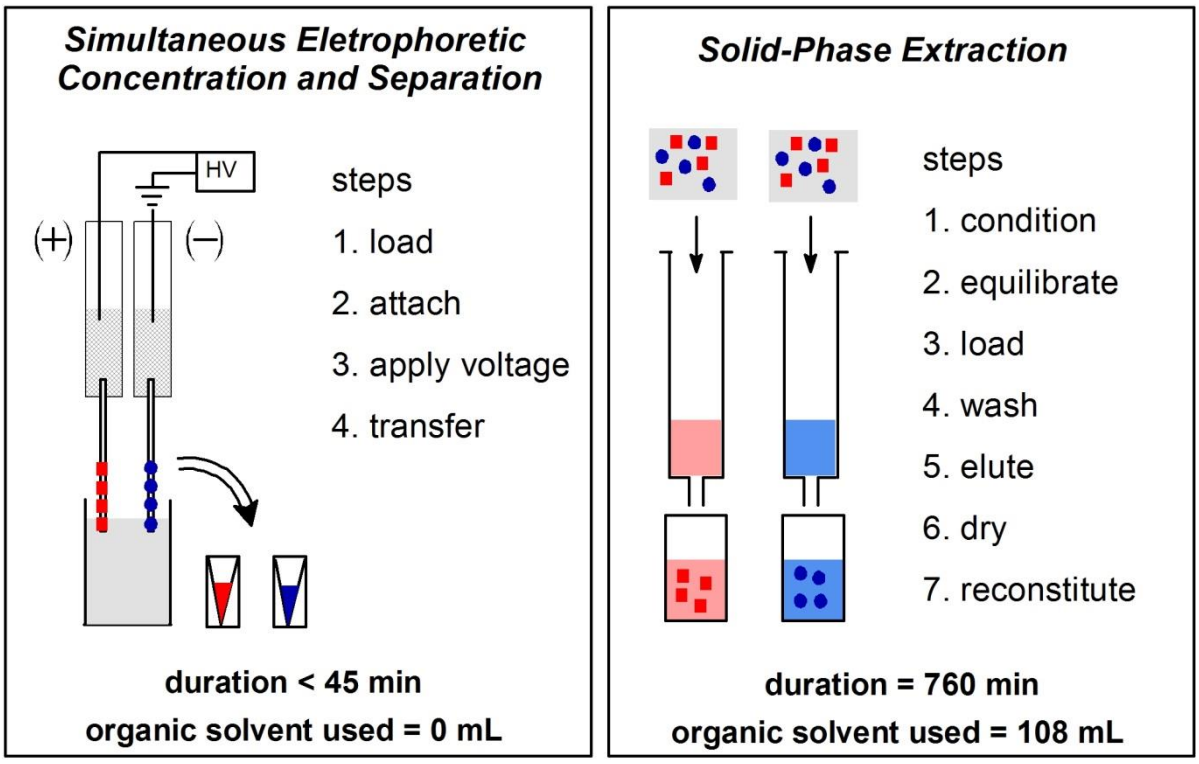
Chapter 5

Green sample preparation for liquid chromatography and capillary electrophoresis of anionic and cationic analytes



Graphical abstract

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5.1 Abstract

A sample preparation device for the simultaneous enrichment and separation of cationic and anionic analytes was designed and implemented in an 8-channel configuration. The device is based on the use of an electric field to transfer the analytes from a large volume of sample into small volumes of electrolyte that was suspended into two glass micropipettes using a conductive hydrogel. This simple, economical, fast, and green (no organic solvent required) sample preparation scheme was evaluated using cationic and anionic herbicides as test analytes in water. The analytical figures of merit and ecological aspects were evaluated against the state of the art sample preparation, solid-phase extraction. A drastic reduction in both sample preparation time (94% faster) and resources (99% less consumables used) was observed. Finally, the technique in combination with high performance liquid chromatography and capillary electrophoresis was applied to analysis of quaternary ammonium and phenoxypropionic acid herbicides in fortified river water as well as drinking water (at levels relevant to Australian guidelines). The presented sustainable sample preparation approach could easily be applied to other charged analytes or adopted by other laboratories.

5.2 Introduction

A general concern of sample preparation is that it is often accompanied by a laborious and resource intensive workflow. Additionally, the use of toxic reagents and appreciable volumes of organic solvents further diminish the eco-friendliness of any analysis. Widely applied sample preparation techniques are solid-phase extraction (SPE) and liquid-liquid extraction (LLE).[1,2] Both techniques rely on the distribution of the analyte between a donor phase or sample solution and an acceptor phase. For instance, in SPE the sample solution is brought in contact with a solid stationary phase. Due to different partitioning of the analyte with the stationary phase, the analyte can be retained selectively and therefore separated from the bulk solution. After elution of the analyte from the stationary phase, the eluate is evaporated and reconstituted with a suitable diluent. The partition or distribution coefficient of the analyte with the acceptor phase is the driving force but also the limiting factor in SPE as well as in LLE. For example, efficient extraction of polar or ionisable analytes which are more soluble in aqueous phases is challenging in LLE and SPE. Thus, the application of an electrical field as a driving force having orthogonal characteristics to adsorption or partition has attracted interest and this approach has been applied to enhance the extraction of ionisable molecules from aqueous samples.[3–15] Selective enrichment of either cationic or anionic molecules can also be accomplished and samples produced from these procedures do not normally require further processing and are often analysed directly.

Herbicides are used to control or eliminate unwanted plant growth. They are used particularly in agriculture to increase productivity. Selective herbicides kill specific targets, whereas the non-selective group destroys all plants in contact with them. Paraquat and diquat belong to the latter class and are prominent examples of the family of cationic quaternary ammonium herbicides. Difenzoquat is in the same family, but is applied as selective weed killer.[16,17] Other examples of selective herbicides are the anionic mono-, di- and tri-chlorophenoxy propionic acids, commonly referred as mecoprop, dichlorprop and fenoprop.

Mecoprop and dichlorprop are widely used as weedkillers in households and agriculture. Together, they are among the most widely used herbicides in the world and are classified with slight to high toxicity. These anionic and cationic herbicides are also highly polar and thus can easily contaminate aquatic ecosystems.[18,19]

Analytical methods capable of detecting low concentrations in large volume water samples require dedicated sample preparation procedures. The different polarities and charges of these herbicides would normally require the use of two parallel SPE or LLE experiments, where one experiment would target the anionic analytes and the other would target the cationic analytes. For example, SPE sorbents containing weak cation- or anion-exchange properties to enrich the anionic and cationic herbicides, respectively, are used commonly. A polymeric SPE sorbent with methanol, dichlormethane and acetronitrile as conditioning and elution solvents has been applied to extract dichlorprop and mecoprop.[20] As with most SPE procedures for large volume samples, large amounts of organic solvent and a tedious workflow were also required for enrichment of quaternary ammonium herbicides from drinking water.[21] Thus, there is a need to introduce green sample preparation methodologies as alternatives to these accepted approaches. Green analytical chemistry approaches target to increase extraction efficiency and/or minimise environmental impact.

Stacking was developed originally for the sole purpose of increasing the detection sensitivity of CE by increasing the sample load, however stacking is also a means of sample preparation which is open for further exploration since it can tremendously concentrate analytes into a narrow zone.[22] We have previously communicated an off-line sample preparation scheme based on stacking by field-enhanced sample injection and demonstrated its use for the selective electrophoretic concentration of anionic analytes.[23] The analytes from a large volume of sample were enriched into a microliter volume of higher conductivity electrolyte that was immobilised inside a micropipette using a hydrogel. Significant analyte enrichment without the use of organic solvents and with limits of detection (LOD) as low as 1

ng/mL after capillary electrophoresis (CE) was obtained. In this work, a new approach to electrophoretic concentration was developed using two micropipettes for the simultaneous concentration and separation of cationic and anionic analytes. The throughput of the approach was increased by implementation in an 8-channel device. Fundamental parameters on this sample preparation called SECS (simultaneous electrophoretic concentration and separation) were investigated using cationic (paraquat, diquat, and difenzoquat) and anionic (mecoprop, dichlorprop and fenoprop) herbicides. SECS was compared to state-of-the-art SPE in terms of analytical performance and environmental considerations. SECS was then optimised and applied to the analysis of herbicides in fortified drinking and river water samples in combination with analysis using high performance liquid chromatography (HPLC) and CE.

5.3 Materials and methods

5.3.1 Reagents and standards

Acrylamide (>99%), potassium persulfate (>99%), ammonium acetate (>99%-wt), sodium phosphate monobasic (NaH_2PO4, >99%), methanol (HPLC grade), acetonitrile (HPLC grade), formic acid (>95%), acetic acid (>99.7%), phosphoric acid (85%), methylene blue), and Ponceau 4R were obtained from Sigma-Aldrich (New South Wales, Australia) and used as delivered. Purified water was from a Milli-Q system (Millipore, MA, USA). Stock electrolyte solutions of 1 mol/L sodium phosphate pH 2.4 and 0.5 mol/L ammonium acetate pH 9 and pH 5 were prepared in purified water. The pH of the stock solutions was adjusted when needed using 1 mol/L sodium hydroxide or 30% ammonium hydroxide solution. The pH and conductivity of solutions were measured using a bench top meter from Sper Scientific (Australia). All stock solutions were sonicated and filtered using a 0.45 μm filter prior to use. Drinking water was collected from a tap and river water from the Derwent River (New Norfolk, Tasmania, Australia).

Paraquat tetrahydrate (99.5%) and difenzoquat methylsulfate (99.5%) were purchased from Supelco (Bellefonte, PA, USA) and diquat monohydrate (99.9%), 2-(4-chloro-2-methylphenoxy)propionic acid (99.6%, mecoprop), 2-(2,4-dichlorophenoxy)propionic acid (99.9%, dichlorprop) and 2-(2,4,5-trichlorophenoxy)propionic acid (97.7%, fenoprop) were obtained from Fluka Analytical (St. Louis, MO, USA) and used without further purification. Analyte stock solutions of 10 mg/mL each in purified water or 50% acetonitrile were prepared and stored at 5-8°C when not in use. The stock analyte mixture comprised 100 μg/mL of both cationic (paraquat, diquat, difenzoquat) and anionic (mecoprop, dichlorprop and fenoprop) herbicides in purified water. Aliquots of this solution were spiked into the sample solution to make up the final concentration.

5.3.2 Hydrogel preparation

Hydrogels were prepared directly in 3 mL capacity polypropylene syringes with the plunger removed from and where the narrower end was sealed with parafilm. A basic hydrogel (total volume = 1.2 mL) was made by mixing 600μL 55%-wt acrylamide (monomer), 120 μL 0.5 mol/L ammonium acetate at pH 9, 420 μL purified water, and 60 μL 5%-wt potassium persulfate (initiator). An acidic hydrogel was prepared using the same conditions except with 120 μL 0.5 mol/L ammonium acetate pH 5 instead of pH 9. The mixture was heated at 60°C for 10 min.[23]

5.3.3 Simultaneous electrophoretic concentration and separation

The scheme for SECS is depicted in Figure 5.3.3.1 and a diagram showing the SECS procedure is found in *5.7 Supporting Information* (SI), Figure S1. Two micropipettes were filled with a solution of ammonium acetate at pH 9 (the acceptor phase for anionic herbicides) or pH 5 (the acceptor phase for cationic herbicides) (SI Figure S1a). The micropipettes containing the acceptor phases were then partially inserted into the bottom of the basic and acidic hydrogels, respectively, contained in the disposable syringe barrels. A platinum wire was attached at the top of each of the hydrogels. The micropipettes were then dipped into the same sample solution

containing anionic and cationic analytes (SI Figure S1b). The sample volume was kept at 20 mL. Voltage was applied with positive electrode at the basic hydrogel and negative electrode at the acidic hydrogel (SI Figure S1c). This produced an electric field to attract the negatively and positively charged herbicides into the pH 9 (red) and pH 5 (blue) acceptor phases inside the micropipette, respectively. The net liquid flow inside the micropipette was zero because of the hydrogel.[24] After application of voltage for the desired time, the entire acceptor solutions were then transferred into separate vials (SI Figure S1d). The pH 5 acceptor phases containing the cationic herbicides were analysed by CE. The pH 9 acceptor phases with the anionic herbicides were analysed by HPLC.

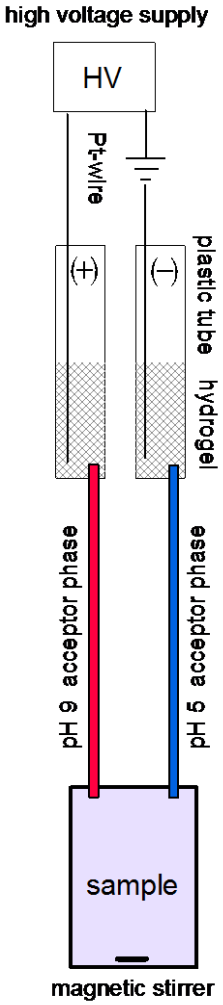

Figure 5.3.3.1. Schematic for SECS.

This experimental set-up was expanded for the simultaneous analysis of up to eight samples with an 8-channel high voltage power supply HVS448-6000D from Labsmith (Livermore, CA, USA) that was capable of providing adjustable voltages of -3 to 3 kV (1 V increments). This expanded set-up is shown in SI Figure S2. The 20 µL micropipettes (length = 6.4 cm and an inner diameter = 0.6 mm) were from Drummond Scientific Company (Broomall, PA, USA), 3 mL disposable plastic syringes were from Terumo (Binan, Laguna, Philippines), and 50 mL capacity polypropylene sample vials were from Sarstedt (Mawson Lakes, SA, Australia). The 10-place magnetic stirrer was from LabCo (Cambridge, TAS, Australia) with stirrer bars (length x width = 3 x 1 mm).

The applied voltage was adjusted based on the conductivity of the sample matrix (2.7, 63.5, and 112.3 µS/cm for purified, drinking and river water, respectively). As a system suitability test, the applied voltage was adjusted such that the observed current was 200–300 µA after 1 min. This avoided bubble formation inside the micropipette which was observed when the current was >400 µA. The applied voltage for purified, drinking and river water was 2.0, 1.0, and 0.5 kV, respectively.

5.3.4 Weak cation-exchange of cationic herbicides

A 12-port vacuum manifold (Visiprep) from Sigma-Aldrich (NSW, Australia) was used for parallel ion-exchange SPE. Cationic herbicides were extracted using polymeric weak cation-exchange SPE cartridges with 1g sorbent mass and 12 mL volume (Strata-X-CW 33u) from Phenomenex (NSW, Australia). The manufacturer's recommended procedure was modified because of the poor concentration factors and repeatability for the highly polar paraquat and diquat. In order to effectively enrich the analytes into the sorbent, a lower flow rate and lower concentration of washing buffer were implemented. The following steps (for one SPE cartridge) were found to be optimal. The sorbent bed was conditioned with 20 mL acetonitrile and then equilibrated with 20 mL 1 mmol/L sodium phosphate pH 7. 20 mL sample solution consisting of 1 mmol/L sodium phosphate pH 7 was loaded at a flow rate of 2 mL/min. The sorbent was

then washed with 20 mL 1 mmol/L sodium phosphate at pH 7 and 20 mL 50% methanol. Finally, elution was at 2 mL/min using 20 mL 5% formic acid in acetonitrile into 50 mL centrifuge tube from Nest (China). The eluates were completely dried overnight at 60 ˚C and 550 mbar and reconstituted in 1 mL 50 mmol/L ammonium acetate pH 5.

5.3.5 Weak anion-exchange of anionic herbicides

Anionic herbicides were extracted using polymeric weak anion-exchange SPE cartridges with 1g sorbent mass and 12 mL volume (Strata-X-AW 33u) from Phenomenex (NSW, Australia). The procedure recommended by the manufacturer was found to be suitable and was as follows. The sorbent bed was conditioned with 20 mL acetonitrile and then equilibrated with 20 mL of 5 mmol/L ammonium acetate pH 6.2. 20 mL sample solution buffered with 5 mmol/L ammonium acetate pH 6.2 was loaded at a flow rate of 2 mL/min. The sorbent was washed with 20 mL of 5 mmol/L ammonium acetate at pH 6.2 and 20 mL methanol. Finally, the anionic herbicides were eluted at 2 mL/min using 20 mL 5% ammonium hydroxide in acetonitrile. The eluates were completely dried overnight at 60 ˚C and 550 mbar and reconstituted in 1 mL 50 mmol/L ammonium acetate pH 9.

5.3.6 Analysis of standards, samples and concentrates

CE and HPLC were used to analyse the cationic and anionic herbicides, respectively. CE was performed on a G1600 Agilent 3D system (Waldbronn, Germany). Fused-silica capillaries (33 cm total length, 24.5 cm to the detection window) were obtained from Molex (Phoenix, AZ, USA). The separation electrolyte was 150 mmol/L sodium phosphate at pH 2.4 and the applied voltage was 10 kV, respectively. The capillary was thermostated at 20 °C, detection was by UV-absorption at 200 nm, and the sample injection was at 50 mbar for 5 s.

The electrophoretic mobility of the herbicides were determined using CE. For the cationic herbicides, the separation electrolyte was 50 mmol/L ammonium acetate at pH 5 and the separation voltage was 20 kV. For the anionic herbicides, the electrolyte was 50 mmol/L

ammonium acetate at pH 9 and voltage was -20 kV. The mobility values for paraquat, diquat, difenzoquat, dichloprop, mecoprop and fenoprop were 4.7 x 10^{-08}, 4.6 x 10^{-08}, 1.9 x 10^{-08}, -2.1 x 10^{-08}, -2.1 x 10^{-08}, and -2.0 x 10^{-08} m^2/V s, respectively.

HPLC was performed on an Agilent 1200 Infinity system (Waldbronn, Germany) using a Dionex Acclaim 120 column (C18, 5µm, 120Å, 4.6 mm diameter and 150 mm length) thermostated to 25 °C. The isocratic mobile phase consisted of 64%-v/v of 25 mmol/L NaH_2PO4 at pH 2.9 and 36%-v/v acetonitrile. The flow rate, injection volume and UV detection was 1 mL/min, 10 µL and 230 nm, respectively.

5.4 Results and Discussion

5.4.1 Optimisation of SECS

The effect of stirring and electrolyte concentration in the acceptor phases were evaluated using purified water as the sample diluent for the herbicides. The acceptor phases were 50 mmol/L ammonium acetate at pH 5 and pH 9. Voltage was at 2 kV for 30 min without and with stirring at 600 rpm. A higher stirring rate was avoided, because this created a vortex. The concentration factor for each analyte is shown in SI Figure S3. The experiments with stirring provided higher factors which were 150-300 and 77-94 for the cationic and anionic herbicides, respectively. Without stirring the concentration factors were 57-106 and 76-83, respectively. The concentration factors were also repeatable when stirring was applied. With stirring, the %RSD values of concentration factors were 5.3-7.0% and 7.8-12.0% for the anionic and cationic herbicides, respectively. The %RSDs without stirring were 36.2-88.5% and 25.2-29.4%, correspondingly. Therefore, stirring is critical to the success of SECS. This was also in agreement with the results from other groups working on electric field driven sample concentration.[12,25] For example, Hirokawa and co-workers observed enhanced transport of analytes in electrokinetic supercharging-CE by sample stirring.[25]

Without stirring, analyte depletion was observed visually with the aid of high mobility cationic (methylene blue) and anionic (Ponceau 4R) dyes) in the top half of the sample solution (data not shown). Thus, the analytes were only accessible for SECS in this top half of the sample. In electrokinetic injection, which is the basis of SECS, there is greater preference for higher mobility ions to enter the pipette. This is also known in CE as the bias occurring with electrokinetic injection.[26] With stirring, the entire sample solution was accessible to electrokinetic injection, thus larger amounts of the high mobility herbicides were introduced. The injection of lower mobility analytes (all anionic herbicides and difenzoquat) was slow and thus the amount of herbicides accessible to SECS was not significantly different with or without stirring. This also explains the different concentration factors observed for the herbicides in SECS with or without stirring.

The concentration of ammonium acetate in the pH 5 and pH 9 acceptor phases was studied at 25, 50 and 100 mmol/L. The concentration of ammonium acetate was the same in both anion and cation acceptor phases during each SECS experiment. Voltage and voltage application time were 2 kV and 30 min. The concentration factor was calculated by dividing the peak area of the sample obtained after sample preparation with the peak area of a standard and then multiplied with the dilution factor. The concentration factors using an acceptor concentration of 25 mmol/L were 59-86 and 59-79 for the cationic and anionic herbicides, respectively. The factors using 50 mmol/L were 150-337 and 18-24 and using 100 mmol/L were 116-190 and 10-12, respectively. Given the above conditions for voltage and voltage application time, the 50 mmol/L acceptor phases were selected for further studies, because they provided the highest averaged concentration factors. In addition, the 50 mmol/L acceptor phases provided a lower running current which offered better stability.

The amount of sample ions concentrated into the acceptor phase depends on the electrophoretic concentration time and the nature of the water sample.[23] The voltage application time in SECS for purified, drinking, and river water was then studied using 50

mmol/L ammonium acetate at pH 5 and 9 as acceptor phases and with sample stirring at 600 rpm. The results are shown in SI Figure S4(a), (b), and (c), respectively. For purified water, the concentration factors of the higher mobility cations (paraquat and diquat) reached a maximum at 30 min. The factors then decreased when the time was >30 min, because a fraction of the concentrated cations migrated out of the micropipette into the hydrogel. For the lower mobility cations and anions (difenzoquat, mecoprop, dichlorprop and fenoprop), the concentration factors gradually increased over 90 min and 60 min, respectively. This was in agreement with electrophoresis, where the concentrated zones of the high mobility ions migrated at higher velocity than the low mobility ions. For the drinking and river water samples, the analytes also reached a maximum concentration factor which decreased as the concentration time was increased. The observed lower concentration factors compared to purified water resulted because the other ions in the sample also entered the acceptor phase. The high mobility ions from the sample matrix (e.g., inorganic salts) entered the acceptor phase first and biased the enrichment of the herbicides. The voltage application time for all the water samples was selected to be 30 min which provided acceptable concentration factors for all analytes.

5.4.2 Environmental considerations (of SECS and SPE)

According to the 12 principles of Green Chemistry and their transformation into analytical chemistry, the main points of concern were the time required and the significant volume of organic solvents used for sample preparation.[27–29] In the SPE of charged herbicides, there were seven steps from conditioning of the cartridge through to elution and reconstitution. For instance, one sample preparation cycle for the anionic herbicides involved the use of 59 mL organic solvents and 41 mL aqueous buffer. The analytes were eluted from the SPE cartridge and the eluate was evaporated to dryness overnight. Evaporation was the most time-consuming step which accounted for more than 12h. A similar procedure was performed for the cationic herbicides and involved the use of 49 mL organic solvents and 51 mL aqueous buffer. SPE for

both groups of herbicides was accomplished simultaneously. Therefore, the total sample preparation time was approximately 12 h 40 min and the total solvent volume was 200 mL (54% was organic solvent). The cost of two SPE cartridges was approximately 30 AUD (15 AUD each).

On the contrary, an SECS cycle for both groups of herbicides took less than 45 min. Four simple steps were involved and these did not require the use of any organic solvents. The total volume of aqueous solution was approximately 2.4 mL. These translate to a reduction in solvent/reagent and time of 99% and 94%, respectively. Additional advantage of SECS was the reduction in running costs. The cost of two micropipettes and two plastic tubes was less than 0.9 AUD.

5.4.3 Analytical figures of merit applied in quality assurance/quality control of the entire procedure

Analytical figures of merit for the entire analytical procedure (sample preparation by SPE or SECS and analysis by HPLC or CE) were determined. The procedures for cations were CE analysis of the reconstituted extract from weak cation-exchange SPE and the cationic concentrate from SECS. The procedures for anions were HPLC analysis of the reconstituted extract from weak anion-exchange SPE and the anionic concentrate from SECS. The parameters used were the calibration range including regression line, squared correlation coefficient (R^2), method detection limit (MDL), method quantitation limit (MQL), precision expressed as repeatability and intermediate precision as well as uncertainty associated with repeatability (U).[30,31]

Linearity and MDLs were obtained by adding the herbicide stock solution to purified water in the range of 10-2000 ng/mL (each concentration was analysed in duplicate). Then, SECS and SPE were performed followed by quantification of the concentrate and reconstituted extract, respectively. The MDLs were calculated at a signal-to-noise ratio (S/N) of 3 based on the electropherogram and chromatogram obtained from the entire procedure. MQLs were

calculated as 3.3x MDL. Precision was evaluated through the percentage relative standard deviation (%RSD) of repeatability and intermediate precision. A sample solution with a concentration of 0.5 µg/mL of each herbicide was used as repeatability sample. Repeatability was calculated from analysis of 6 sample treatments during one day (%RSD, n=6). Intermediate precision was calculated from analysis of 6 sample treatments, 3 treatments per day for 2 days (%RSD, n=6). Uncertainty associated with repeatability (U) was calculated according to equation (1)

$$U = k * \frac{SD}{\sqrt{n}} \quad (1)$$

where k is the coverage factor (k = 2 at 95% confidence interval), SD is the standard deviation, and n is the number of measurements.

5.4.4 Comparison of analytical figures of merit (SECS and SPE)

Table 5.4.4.1 shows the analytical figures of merit and concentration factors for the analysis of cations and anions by CE and HPLC, respectively, with sample preparation by (a) SECS and (b) SPE. The linear range for both sample preparation techniques combined with HPLC or CE was similar and was approximately two orders of magnitude. In terms of sensitivity, the analytical procedures were comparable. However, electrophoretic concentration of the cationic herbicides provided MDLs that were 10-times lower than for the same analytes extracted by SPE. This resulted from the fast migration of the cationic herbicides into the micropipettes and therefore higher enrichment factors. In SPE, the enrichment is limited by the capacity of the sorbent and the sample loading, and volume after reconstitution.

Table 5.4.4.1 Analytical figures of merit and concentration factors obtained for herbicides in purified water after treatment with (a) SECS and (b) SPE and analysis by CE (cationic herbicides) and HPLC (anionic herbicides).

(a) SECS	**Linear range (ng/mL)**	**Regression (y=)**	**R^2**	**MDL[a] (ng/mL)**	**MQL[b] (ng/mL)**	**Repeatability[c], (%RSD, n=6)**	**Intermediate precision[c], (%RSD, n=6)**	**Concentration factor[d]**
Paraquat (CE)	10 - 2000	0.1658x + 1.0006	0.999	0.5	1.7	2.4%	3.0%	313
Diquat (CE)	10 - 2000	0.1348x + 0.6892	0.999	0.5	1.7	5.3%	7.1%	337
Difenzoquat(CE)	10 - 2000	0.1372x - 1.1431	0.999	0.5	1.7	8.8%	11.5%	150
Dichloprop (HPLC)	20 - 2000	1350.5x + 2958.3	1.000	5.0	16.7	3.0%	11.2%	23
Mecoprop (HPLC)	20 - 2000	1369.9x - 3791.8	1.000	5.0	16.7	3.1%	11.3%	24
Fenoprop (HPLC)	20 - 2000	1101.5x - 2539.9	1.000	5.0	16.7	4.5%	12.0%	18
(b) SPE	**Linear range (ng/mL)**	**Regression (y=)**	**R^2**	**MDL[a] (ng/mL)**	**MQL[b] (ng/mL)**	**Repeatability[c], (%RSD, n=6)**	**Intermediate precision[c], (%RSD, n=6)**	**Concentration factor[d]**
Paraquat (CE)	20 - 2000	7.6527x - 0.214	0.997	5.0	16.7	3.2%	9.2%	18
Diquat (CE)	20 - 2000	7.1297x - 0.2244	0.996	5.0	16.7	7.5%	10.0%	16
Difenzoquat (CE)	20 - 2000	13.922x + 0.5264	1.000	5.0	16.7	3.6%	4.8%	20
Dichloprop (HPLC)	20 - 2000	335.48x - 9.9673	0.980	5.0	16.7	2.1%	2.6%	18
Mecoprop (HPLC)	20 - 2000	329.73x - 14.365	0.997	5.0	16.7	6.3%	7.0%	17
Fenoprop (HPLC)	20 - 2000	516.25x - 19.101	0.998	5.0	16.7	1.7%	2.7%	17

[a] MDL was calculated based on a S/N = 3.
[b] MQL = 3.3x MDL.
[c] At a concentration of 0.5 µg/mL.
[d] The concentration factor was calculated by dividing the peak area obtained from the concentrate or extract by the corresponding peak area of a standard sample and then multiplied with the dilution factor. SECS conditions were 2.0 kV and 30 min (a).

The repeatability of the analytical procedure with SECS was 2.4 - 8.8%. The reportable values which considered the calculated U from the analysis of the repeatability sample (500 ng/mL each) for paraquat, diquat, difenzoquat, dichlorprop, mecoprop, and fenoprop were 500 ± 10, 500 ± 22, 500 ± 36, 500 ± 12, 500 ± 20, 500 ± 19 ng/mL, respectively. With SPE, the repeatability values were slightly better at 1.7 - 7.5%. The reportable values were 500 ± 13, 500 ± 31, 500 ± 15, 500 ± 9, 500 ± 26, 500 ± 7 ng/mL, correspondingly. The intermediate precision was also slightly better with SPE (2.6 - 10.0%) compared to SECS (3.0 - 12.0%).

The maximum value for concentration factor is the volume of the sample solution divided by the volume used for reconstitution (SPE) or as acceptor phase (SECS). In this work, the maximum values for SECS and SPE are 1000 (= 20 mL/20 μL) and 20 (= 20 mL/1 mL), respectively. Higher concentration factors for SPE could be obtained by increasing the sample volume and/or decreasing the volume for reconstitution. The latter was difficult and produced poor repeatabilities and thus is generally not applicable. The sample volume was increased to 1000 mL in order to have the same maximum factor of 1000. However, it took more than 8 h (at a flow rate of 2 ml/min) to load the sample in the SPE cartridge. The actual concentration factors for SECS were 150 - 337 and 18 - 24 for the cationic and anionic herbicides, respectively. For SPE, the concentration factors were 16 - 20 and 17 - 18 for the cationic and anionic herbicides, correspondingly.

SECS is a non-exhaustive technique since it is extremely difficult to transfer all the analytes from a large volume of sample into a small volume of acceptor phase in a single step and within a short period of time. However, the large volume ratio allowed higher concentration factors (larger than one order of concentration magnitude) compared to SPE.

5.4.5 Application of SECS to drinking and river water

The analytical figures of merit and concentration factors are shown in SI Table S1. The %RSD for repeatability/intermediate precision of drinking and river water were 2.1 - 8.8%/6.1 - 10.3% and 3.2 - 9.1%/5.8 - 12.1%, respectively. The concentration factors were 32 - 124 and 31 - 83, respectively. The correlation coefficients (R^2) for calibration linearity for drinking and river water samples were acceptable at 0.997 - 1.000 and 0.998 - 0.999, respectively. MDLs and MQLs were 5 and 17 ng/mL, respectively, for both sample matrixes. The health-related guideline values in Australia for drinking water for paraquat, diquat, difenzoquat, mecoprop, dichlorprop, and fenoprop are 20, 7, 100, 10, 100, and 40 ng/mL.[32] Thus, SECS could easily be used for drinking water monitoring. The use of an internal standard or standard addition method is a possible strategy for accurate analyte concentration determination.

It is noted, the reported MDLs for the anionic herbicides in Table 5.4.4.1 and Table S1 were 5 ng/mL for all analytes and in all three water matrices, although the concentration factors of the analytes in purified water were ca. 5-fold lower. The reason for this discrepancy was that two HPLC systems were used for the analysis of the water samples and thus, the MDL could not be compared because of differing levels of baseline noise.

The concentration factors behaved differently for drinking and river water compared to purified water. The values were highest for cationic herbicides in purified water. For the complex water samples, the values were highest for the anionic analytes. This reversal behaviour in concentration factor is attributed to the sample matrix. The presence of high mobility cations in drinking and river water samples reduced the efficiency of electrokinetic injection for the cationic herbicides. In SECS, ions from the acceptor electrolyte are replaced by ions from the sample. The high mobility cations in the sample competed with the target analyte ions.

5.5 Conclusions

Sample stacking which was originally used to overcome the poor detection sensitivity in CE was applied here as a simple, fast and environmentally-friendly sample preparation method. Cationic and anionic analytes were simultaneously concentrated and separated using a simple set-up in a sample preparation device capable of 8 parallel experiments. With the use of an electric field and a large volume ratio between sample and acceptor phase, significant concentration factors in a short period of time were obtained. No organic solvents were involved and the concentrate was directly compatible with liquid phase analytical separation techniques. The method (entitled SECS) compared to SPE was also easier to optimise and gave larger concentration factors for some analytes. From the environmental point of view, SECS was a faster and greener sample preparation technique for large volume aqueous samples. The approach could easily be applied to other charged samples or adopted by other chemists anywhere. The potential of this approach is currently being investigated for the analysis of drugs (tricyclic antidepressants, β-blockers, penicillins, etc.), pesticides (glufosinate, glyphosate) and other bioactive compounds in environmental as well as food samples (e.g., juices, beer and alcoholic beverages). The use of other chemical analysis techniques such as mass spectrometry is also being explored.

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5.7 Supporting information

5.7.1 Figures

Figure S1. Diagram for SECS procedure.

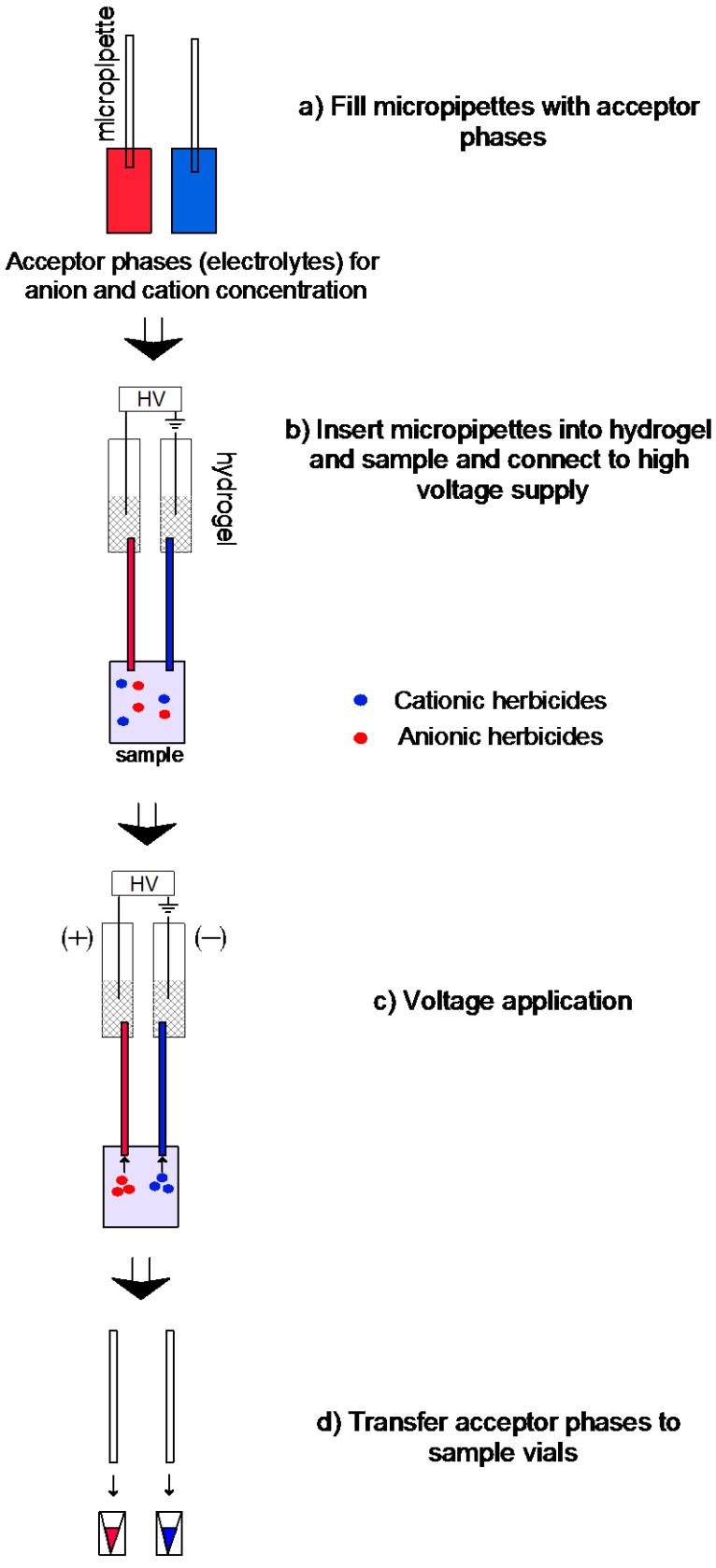


Figure S2. Experimental set-up for simultaneous electrophoretic concentration and separation (SECS) of charged analytes.

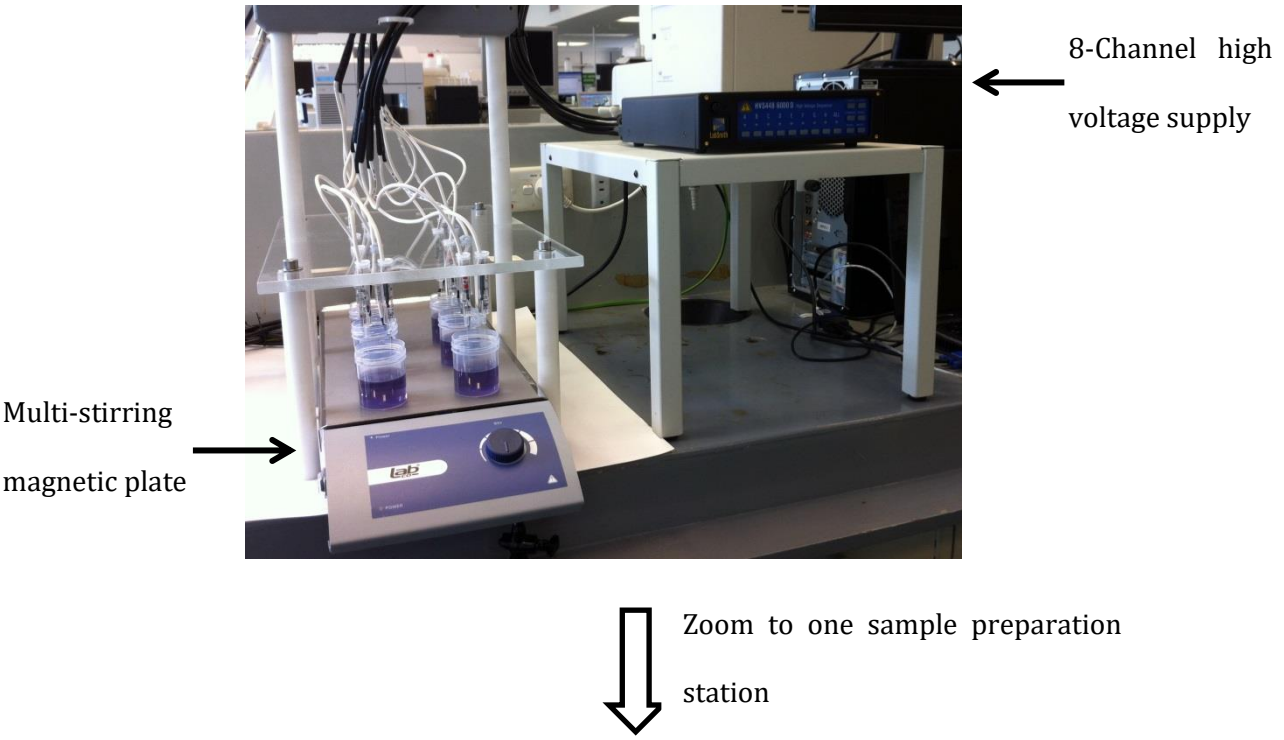
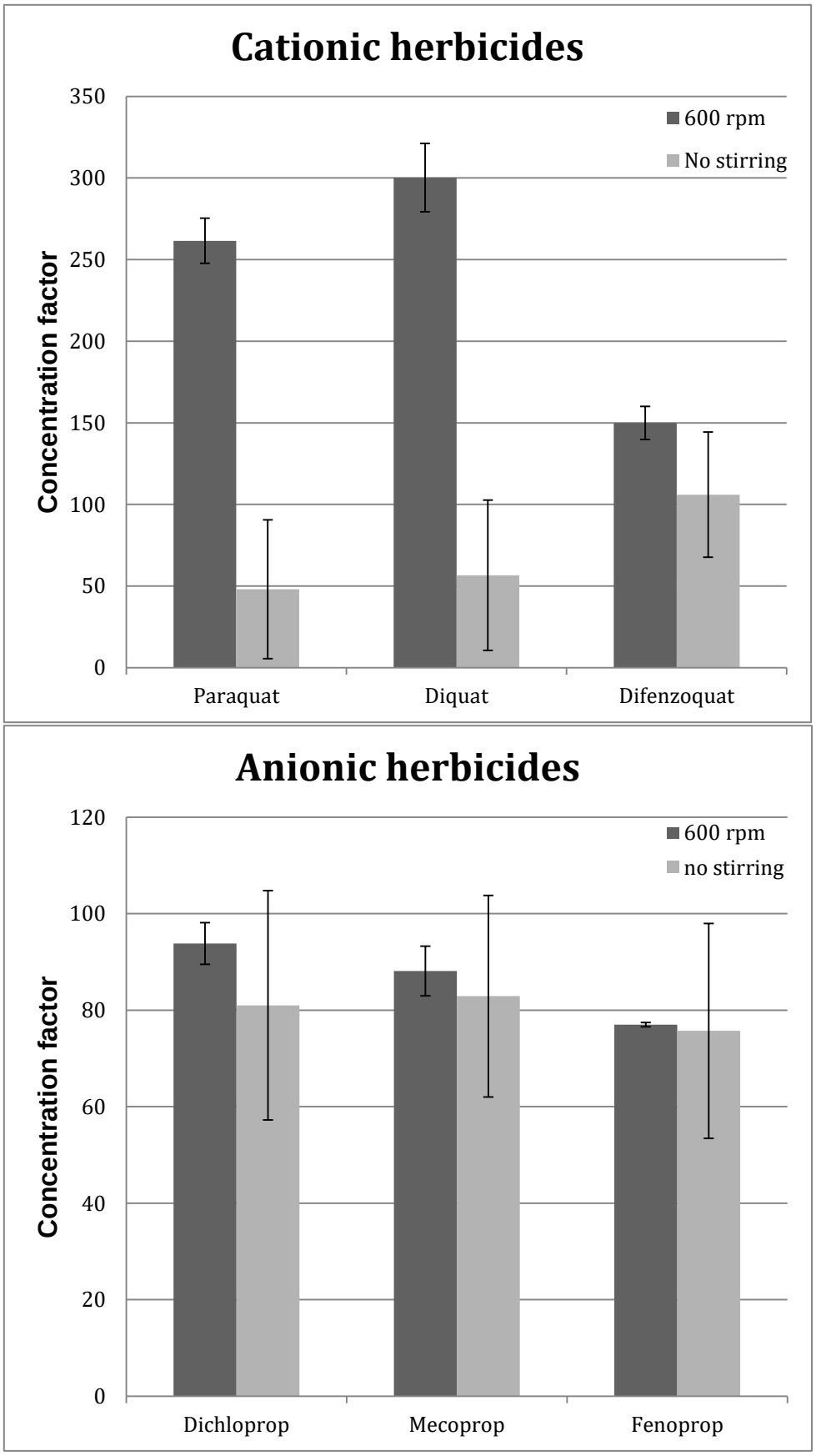
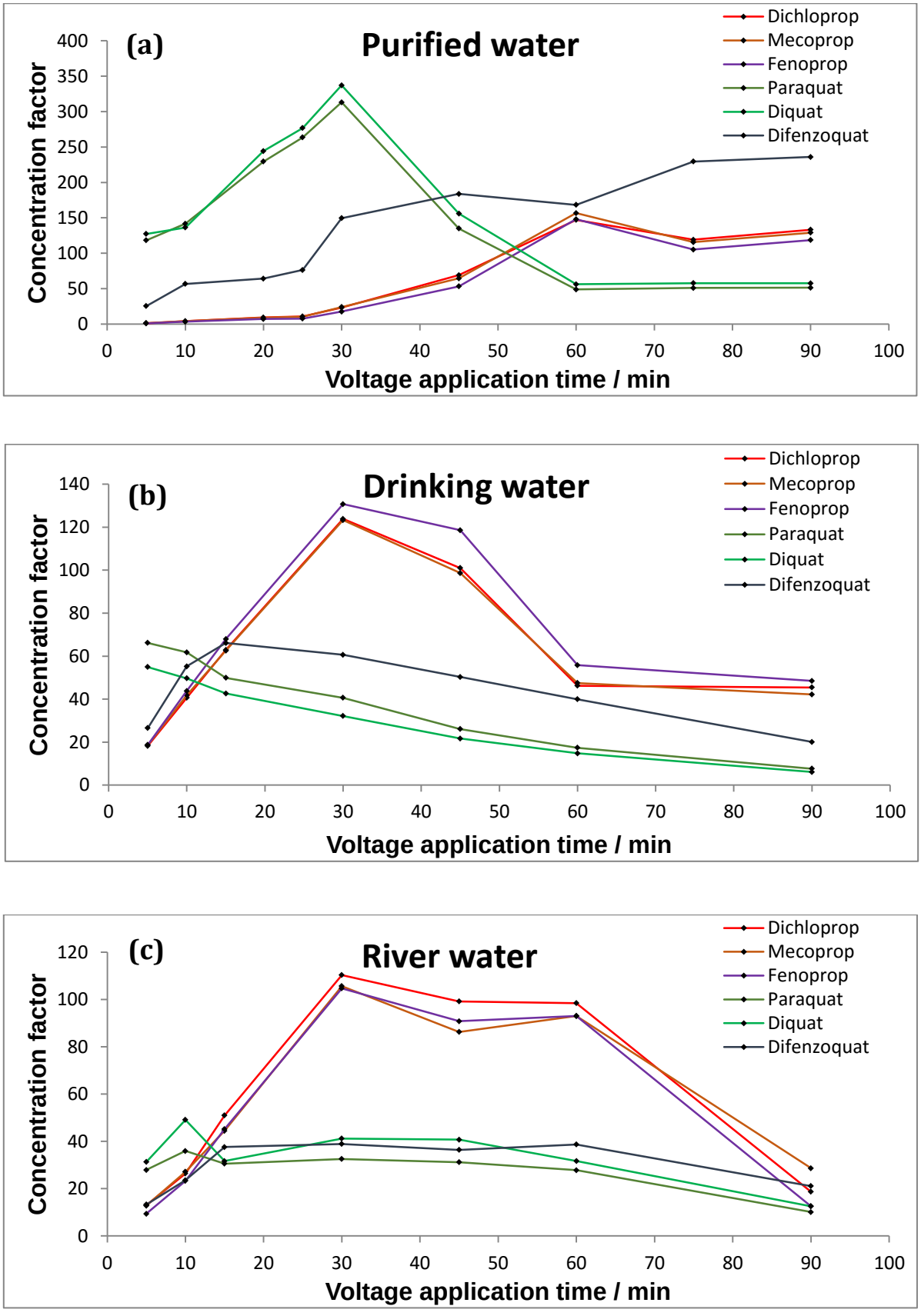


Figure S3. Effect of sample solution stirring on the concentration factor of the six herbicides.



The error bars indicate the variation (% RSD of peak area, n=5) in concentration factor with and without stirring of the sample solution.

Figure S4. Effect of voltage application time on concentration factor for (a) purified, (b) drinking and (c) river water samples.



5.7.2 Table

Table S1. Analytical figures of merit and concentration factor for (a) drinking and (b) river water

(a)	Linear range (ng/mL)	Regression (y=)	R^2	MDL[a] (ng/mL)	MQL[b] (ng/mL)	Repeatability[c], (%RSD, n=6)	Intermediate precision (%RSD, n=6)	Concentration factor[d]
Paraquat	20 - 2000	19.139x - 0.0017	0.999	5.0	16.7	2.7%	6.0%	41
Diquat	20 - 2000	16.94x - 0.592	0.997	5.0	16.7	2.1%	8.2%	32
Difenzoquat	20 - 2000	41.01x - 0.9635	0.998	5.0	16.7	4.2%	7.9%	61
Dichloprop	20 - 2000	1E+06x + 31911	0.999	5.0	16.7	5.0%	6.1%	124
Mecoprop	20 - 2000	2E+06x + 6210.9	1.000	5.0	16.7	8.8%	10.3%	123
Fenoprop	20 - 2000	1E+06x + 9124.1	0.999	5.0	16.7	8.7%	9.0%	131

(b)	Linear range (ng/mL)	Regression (y=)	R^2	MDL[a] (ng/mL)	MQL[b] (ng/mL)	Repeatability[c], (%RSD, n=6)	Intermediate precision (%RSD, n=6)	Concentration factor[d]
Paraquat	20 - 2000	14.556x + 0.0412	0.998	5.0	16.7	3.2%	5.8%	31
Diquat	20 - 2000	13.153x + 0.0243	0.998	5.0	16.7	4.3%	6.1%	32
Difenzoquat	20 - 2000	26.662x + 0.235	0.997	5.0	16.7	3.9%	6.9%	38
Dichloprop	20 - 2000	371945x + 6909.8	0.998	5.0	16.7	4.8%	10.9%	83
Mecoprop	20 - 2000	544695x + 5330.5	0.999	5.0	16.7	4.3%	11.5%	75
Fenoprop	20 - 2000	311143x - 788.42	0.998	5.0	16.7	9.1%	12.1%	70

[a] MDL was calculated based on a S/N = 3

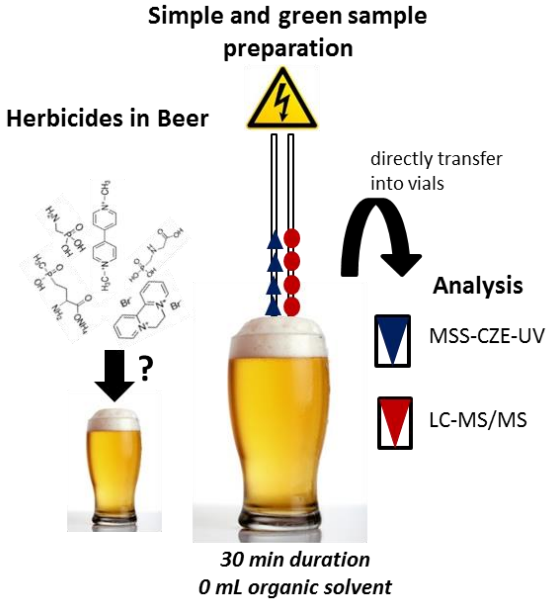
[b] MQL = 3.3x MDL

[c] At a concentration of 0.5 µg/mL.

[d] The concentration factor was calculated by dividing the peak area obtained from the concentrate by the corresponding peak area of a standard sample and then multiplied with the dilution factor. The electrophoretic concentration conditions were 1.0 kV and 30 min (a), and 0.5 kV and 30 min (b).

Chapter 6

Simultaneous electrophoretic concentration and separation of herbicides in beer prior to stacking-capillary electrophoresis-UV and liquid chromatography-mass spectrometry



Graphical abstract

*All of this research contained in this chapter has been published as A. Wuethrich, P.R. Haddad, J.P. Quirino, Simultaneous electrophoretic concentration and separation of herbicides in beer prior to stacking-capillary electrophoresis-UV and liquid chromatography-mass spectrometry. *Electrophoresis*. 37, 1122-1128**, 2016**.

6.1 Abstract

Simultaneous electrophoretic concentration and separation (SECS) was used as a simple and environmental-friendly sample preparation strategy for herbicides in beer samples. An electric field was used to facilitate the separation and concentration of the analytes based on their charge from a 20 mL sample of diluted beer into two separate 20 µL aliquots of an acceptor electrolyte housed inside a micropipette. The anionic organophosphate and cationic quaternary ammonium herbicides were concentrated in the anodic and cathodic pipette, respectively. Under optimized conditions, SECS was completed in 30 min at an applied voltage of 150 V which provided analyte concentration factors of up to 90. After sample preparation, the SECS-concentrate of cationic and anionic herbicides was analysed by stacking-capillary electrophoresis with UV detection and also by liquid chromatography-mass spectrometry, respectively. The method detection limit for the diluted and undiluted sample was as low as 3 ng/mL and 15 ng/mL, respectively. The method was linear over two orders of concentration with repeatability and intermediate precision values of better than 5.8 and 7.0 %RSD, respectively. Accuracy values were between 91.0-115.1 %.

6.2 Introduction

Non-selective herbicides are important for agricultural productivity and are used globally. Common examples of these pesticides are paraquat, diquat, glufosinate and glyphosate. The entry of these chemicals into the environment is critical because of potential contamination of food and water sources. The four herbicides are very polar and show low to high toxicity to mammals. The EU commission has set a lower limit of analytical determination of herbicides in food and beverages to assure food safety.[1] The values for paraquat, diquat, glufosinate, and glyphosate are 0.02, 0.1, 0.1, and 0.1 mg/kg, respectively. The analysis of polar and highly water soluble compounds requires dedicated sample preparation as well as appropriate analytical separation and detection techniques. Also, low analyte concentrations are often found in typical samples and this has recently raised greater concern about the accuracy of the analysis.[2]

A popular approach for the determination of the negatively charged organophosphate herbicides is by LC. However, glyphosate, glufosinate and their metabolites contain only weak chromophores, thus UV-detection at wavelengths higher than 200 nm requires derivatisation or the use of alternative detectors. The hyphenation of LC with MS provides an useful alternative for detection of non-UV active molecules. LC-MS analysis has been performed for the determination of glyphosate, glufosinate and aminomethylphosphonic acid (AMPA, metabolite of glyphosate) in beer and barley tea[3], drinking, surface, and ground water[4], and in biological samples[5,6]. Determination of the same analytes was also achieved using CE-UV after analyte derivatization or CE with chemoluminescence or contactless conductivity detection from soil[7], water samples[8–10], human serum[11], and soybean[12].

Common analytical strategies to analyse positively charged quaternary ammonium herbicides include chromatographic or electrophoretic separation. The former is performed predominantly on ion-exchange columns or by reversed-phase liquid chromatography using ion-pair agents added to the mobile phase. Although the herbicides are suitable for UV-

detection, MS has been the most prevalent detection method employed. The determination of paraquat and diquat in environmental and agricultural samples has been conducted by LC-MS for water samples[13–15], irrigation water of crop fields[16], low-moisture food crops[17], and beer and malt samples[18]. The toxicity and wide availability of these analytes has also made them a focus in forensic and metabolic research.[19–21] These herbicides in food and environmental samples were also analysed by CE with stacking being used to improve detection sensitivity.[22–24]

Environmental and food samples usually constitute a complex sample matrix which can distort the analytical result. Sample preparation methods, such as liquid-liquid extraction (LLE) and SPE, are used commonly to clean-up the sample and/or concentrate the target analytes. In LLE, an analyte in an aqueous matrix can be transferred into a water-immiscible phase when the solubility of the analytes is higher in the extractant than in the aqueous phase. This approach is generally not applicable for highly polar analytes, such as the organophosphate and quaternary ammonium herbicides. In SPE, partitioning of the analyte between a stationary and a liquid phase is responsible for the analyte extraction. For the polar and charged herbicides, mixed-mode or ion-exchanger phases must be used and usually a number of different types of SPE cartridges are required to extract a range of analytes present in the same sample. Careful optimization of the SPE-procedures is crucial in order to avoid loss of the analyte (e.g., during sample loading and washing). Furthermore, substantial resources (e.g., organic solvents) are involved and the evaporation of the solvent from the final extract can often be time-consuming.

We recently reported a simple, rapid, and green sample preparation technique for simultaneous electrophoretic concentration and separation (SECS) which uses an electric field to enrich and separate negatively and positively charged analytes.[25] The analytes from a large volume of sample (20 mL) can be transferred into two very small volumes of electrolyte (20 µL) that were trapped inside two micropipettes using a conductive hydrogel. The tips of the pipettes were dipped into the stirred sample while the other end of each micropipettes was attached to two separate hydrogel plugs that were housed in plastic tubes. A voltage was

applied at one of the hydrogels while the other hydrogel was grounded. The acceptor electrolyte was chosen so that it was directly compatible for the analytical techniques to be used for the final analysis. Using a voltage application time of 30 min, high analyte concentration factors of more than 300 were obtained, providing method detection limits (MDL) as low as 0.5 ng/mL.

In the present study, we have applied SECS, followed by analysis using LC-MS/MS and stacking-CE-UV, for the determination of organophosphates and quaternary ammonium herbicides in beer. In SECS, the concentration and pH of the acidic and basic acceptor electrolytes were studied. The voltage application time was optimized to achieve a compromise between concentration factors (CF) or enrichment factors for the range of analytes studied. LC was performed with a porous graphitic column and MS/MS detection was used for the negatively charged organophosphate herbicides glyphosate, glufosinate and AMPA. This method was adapted from EU Reference Laboratories for Residues of Pesticides with some modification in the gradient separation and MS/MS parameters.[26] Two-step stacking in CE-UV was performed by employing sweeping and micelle to solvent stacking (MSS) for paraquat and diquat.[27,28] This stacking approach was chosen because the acceptor electrolyte from SECS was conductive and was thus directly applicable for sweeping-MSS. In sweeping, analyte focusing is facilitated by the use of micelles and the sample must be free of the micelles.[29] In MSS, focusing was achieved by the reversal of the effective electrophoretic mobility of the analyte by the use of organic solvent and micelles.[30] In two-step stacking, the injection length of the micellar solution, sample solution, and organic solvent phase were investigated. Under optimized conditions, the analytical performance of the entire analytical methodology, including the measurement of accuracy by the standard addition method, was also evaluated.

6.3 Materials and methods

6.3.1 Reagents and stock solutions

Acrylamide (>99%), potassium persulfate (>99%), ammonium acetate (>99%-wt), methanol (HPLC grade), acetonitrile (HPLC grade), acetic acid (>99.7%), phosphoric acid (85%), and SDS (>98.5%) were obtained from Sigma-Aldrich (New South Wales, Australia) and used as delivered. Purified water was from a Milli-Q system (Millipore, MA, USA). Stock electrolyte solutions of 1 mol/L sodium phosphate at pH 2, 0.5 mol/L ammonium acetate at pH 9 and pH 5, and 0.2 mol/L SDS were prepared in purified water. The 1 mol/L sodium phosphate at pH 2 stock electrolyte solution was prepared by titration of 1 mol/L phosphoric acid with 5 mol/L sodium hydroxide solution. The pH of the other stock solutions was adjusted when needed using 1 mol/L sodium hydroxide, 30% ammonium hydroxide solution or acetic acid. The pH and conductivity of solutions were measured using a bench top meter from Sper Scientific (Scottsdale, AZ, USA). All stock solutions were sonicated and filtered using a 0.45 μm filter prior to use. Beer (lager type, alcohol content was 3.5 % v/v) was bought from a local store, diluted five times in purified water and degassed by using a VWR symphony™ sonication (VWR International, Murarrie, Queensland, Australia) for 10 min before use.

Paraquat tetrahydrate (99.5%, paraquat) was purchased from Supelco (Bellefonte, PA, USA) and diquat monohydrate (99.9%, diquat), (aminomethyl)phosphonic acid (99%, AMPA), glyphosate (99.9%), and glufosinate ammonium (98.3%, glufosinate) were obtained from Sigma-Aldrich (Seelze, Germany). Analyte stock solutions of 2 mg/mL each in purified water were prepared and stored at 5-8°C when not in use. The stock analyte mixtures comprised 1 mg/mL of the cationic (paraquat, diquat) or anionic (AMPA, glyphosate, glufosinate) herbicides in purified water. Aliquots of these solutions were spiked into the sample solution to make up the final concentration.

6.3.2 Simultaneous electrophoretic concentration and separation of cationic and anionic herbicides

The experimental procedure is described and shown in Chapter 5. The preparation of the hydrogel and SECS materials are found in Section 6.7 Supporting Information (SI). Briefly,

the procedure for SECS was the following. Two micropipettes were filled with a solution of ammonium acetate at pH 9 (the basic acceptor electrolyte for glufosinate, glyphosate, AMPA) or pH 5 (the acidic acceptor electrolyte for paraquat and diquat). One end of the each pipette was partially inserted into the narrower end of the syringe barrel containing the acidic or basic hydrogel and the other end was dipped into the sample solution. The volume of the sample was 20 mL. A Pt-electrode was attached to each hydrogel and connected to the high voltage supply. Then, voltage with positive or negative polarity was applied to produce an electric field (see Section 6.4.1). This caused the migration and separation of anions and cations from the sample into the acceptor electrolytes, depending on the analyte charge. The anions (glufosinate, glyphosate and AMPA) and cations (paraquat and diquat) were attracted towards the positive and negative electrodes, respectively. The hydrogel at the end of each micropipette resulted in a zero net-flow of electrolyte inside the micropipette and supported the current during voltage application.[31] The sample solution was stirred throughout the whole experiment. After SECS, the micropipettes were removed from the apparatus and their entire contents of basic and acidic acceptor electrolyte were transferred into separate vials for analysis by sweeping-MSS-CZE-UV (paraquat and diquat) or LC-MS/MS (AMPA, glyphosate, glufosinate), respectively.

The applied voltage for SECS was adjusted based on the current during voltage application, so that the observed value was 200–300 μA after 1 min of voltage application. This avoided bubble formation inside the micropipette, which was typically observed when the current was >400 μA. The applied voltage for 5-fold diluted beer using acceptor electrolyte concentrations of 25, 50, 75, 100, 150, and 200 mmol/L ammonium acetate was 500, 400, 250, 150, 100 and 85 V, respectively. The concentration of acceptor electrolyte in both micropipettes was the same.

6.3.3 Sweeping-MSS-CZE-UV method for determination of paraquat and diquat

Standards and cationic SECS concentrates were analysed on a G1600 Agilent 3D CE system (Waldbronn, Germany). Fused-silica capillaries (60 and 51.5 cm total length and length

to the detection window, respectively) were obtained from Molex (Phoenix, AZ, USA). The capillary was operated at 20 °C, using UV-detection at 200 nm, and a separation voltage of 22 kV. The sample injection lengths were calculated using the CE Expert software (Beckman-Coulter, USA). The background electrolyte (BGE) was 150 mmol/L sodium phosphate at pH 2. Typical injection was performed for 5 s at 50 mbar. New capillaries were conditioned for 15 min with 0.2 mol/L sodium hydroxide, 5 min purified water and 15 min BGE. The between-run conditioning was performed using 0.5 min 0.2 mol/L sodium hydroxide, 0.5 min purified water and 3 min BGE. Under optimised conditions, the injection regimen was as follows. First, a micellar solution containing 10 mmol/L SDS in 140 mmol/L sodium phosphate at pH 2 was injected at 50 mbar for 150 s. Second, the sample was injected at 50 mbar for 120 s. Third, a 30% acetonitrile solution was injected at 50 mbar for 10 s before the separation voltage was applied.

6.3.4 LC-MS/MS method for determination of glyphosate, glufosinate and AMPA

The method was adapted from EU Reference Laboratories for Residues of Pesticides.[26] Details to the method and parameters are found in the Section 6.7.3 of SI.

6.4 Results and discussion

6.4.1 Study of the acceptor electrolyte concentration for SECS

The enrichment of the analytes inside the micropipettes follows the principle of field-enhanced or amplified sample injection applied in CE to improve detection sensitivity.[32,33] The analyte enrichment is favoured by an increased conductivity ratio between the sample solution and acceptor electrolyte. However, in SECS a compromise between the acceptor electrolyte concentration and the system current during voltage application has to be chosen for stable operation (e.g., to avoid bubble formation inside the micropipettes due to water electrolysis).

Acceptor electrolyte concentrations of 25, 50, 75, 100, 150, and 200 mmol/L were investigated. The applied voltages were 500, 400, 250, 150, 100 and 85 V, correspondingly. These voltages were lower than the EC voltages in Chapter 3-5 (i.e., 500 – 2000 V), because the conductivities were higher of both the diluted beer sample and acceptor phase. The sample solution was 5-fold diluted beer spiked to 100 ng/mL with the analyte stock solution. SECS was performed for 10, 20, 30, and 50 min (in duplicate). After SECS, the anionic and cationic concentrates were analysed by LC-MS/MS and a sweeping-MSS-CE method using a sample injection of 60 s at 50 mbar. These injection parameters for the stacking-CE method provided sufficient sensitivity for analyte quantification in the SECS-concentrates. The CF for each analyte was then plotted over time. The CF was calculated by the peak area of the SECS concentrate divided by the peak area of a standard and multiplied by the dilution factor. The calculation of the CF for the cationic herbicides was with migration time corrected peak areas (peak area/migration time). In CE, peak area correction is necessary because the analytes do not migrate with the same velocity.

SI Figure S1 shows the effect of the acceptor electrolyte concentration on the CF depending on the voltage application time. The CFs for paraquat and diquat using an acceptor electrolyte of 25, 50, 75, 100, 150, and 200 mmol/L were 19-29, 16-27, 41-81, 25-90, 19-70, and 19-73, respectively, for 10-50 min voltage application time. The CFs for AMPA, glyphosate and glufosinate were 1-15, 1-14, 1-14, 1-11, 1-5, and 1-4, correspondingly. The CF of paraquat, diquat, glufosinate and glyphosate increased with the 30-40 min and then decreased with the 50 min voltage application time. The CF of AMPA was not strongly affected by the acceptor electrolyte concentration. The low CF values for AMPA were also prone to more variation from experiment to experiment. It is noted, the averaged CF value at 30 min voltage application time for AMPA and the 150 mM acceptor phase was 0.4 which was rounded down to 0 in Figure S1 (c). The CFs of the anionic herbicides were lower than for the cationic analytes. This was a consequence of the acidity of the diluted beer (pH 3.5) and interference from the sample matrix. The low pH of the sample resulted in the low electrophoretic mobilities for the anionic analytes.

There was also bias to electrokinetic injection in favour of small inorganic anions (in the sample matrix) with higher electrophoretic mobilities than the anionic herbicides. The highest averaged CF of all five analytes was obtained using an acceptor electrolyte concentration of 100 mmol/L ammonium acetate, an applied voltage of 150 V and a voltage application time of 30 min. Using these conditions, the effect of the pH of the acceptor electrolyte on the CF was studied. First, experiments were performed using the acidic and basic acceptor electrolytes for the enrichment of the cationic and anionic herbicides, respectively. In the second set of experiments, the acceptor electrolytes were reversed, so that the acidic and basic electrolytes were used for enrichment of the anions and cations, respectively. The CFs were better in the first configuration where the acidic (pH 5) and basic (pH 9) acceptor electrolyte was used for the extraction of cationic and anionic herbicides, respectively. The first configuration was then used for further investigations.

6.4.2 LC-MS/MS method linearity

The modified LC-MS/MS method used in this study was evaluated in terms of linear range using standard solutions and acceptor electrolytes as diluent. The linear range was determined with analyte concentrations of 0.05, 0.1, 0.5, 1.0, 5.0, 10.0, 20.0, and 50.0 µg/mL (in duplicate). AMPA, glufosinate, and glyphosate were linear from 0.1-20 µg/mL with a coefficient of determination (R^2) of 0.996-1.000.

6.4.3 Sweeping-MSS-CE-UV method optimization

Optimization of the method was performed using a 1 µg/mL analyte solution prepared with acceptor electrolyte as diluent, a micellar solution of 140 mmol/L SDS in 10 mmol/L sodium phosphate at pH 2, and an organic solvent phase of 30% (v/v) acetonitrile in purified water. All solutions were injected at a pressure of 50 mbar. In sweeping-MSS, the sensitivity enhancement of the method is proportional to the sample injection length. However, longer analyte injections can result in broad and unresolved peaks. Thus, parameters affecting the

analyte focusing by sweeping-MSS need to be optimized. Typically, these are the injection length of the analyte solution, the micellar solution and organic solvent phase.

The injection length of the analyte solution as a percentage of the capillary length to the detection window/injection time was studied for 7.6%/60 s, 11.3%/90 s, 15.1%/120 s, 18.9%/150 s, 22.7%/180 s, and 26.5%/210 s. The injection regimen was the following. First, the micellar solution was injected for 120 s, followed by the analyte solution and finally the organic solvent phase for 10 s. For injections of the analyte solution of $\geq$ 150 s, unresolved and broad peaks were obtained for paraquat and diquat. No improvement in peak shape was observed when the injections of the micellar solution and/or organic solvent phase were increased to 150 and 15 s, respectively. Thus, an analyte injection time of 120 s was used to study the injection lengths of the micellar solution. The micelles are responsible for the reversal of the apparent mobility of the analytes and are thus important for the enrichment process.[30] Injection times for the micellar solution of 90, 120, 150, and 180 s were investigated and 150 s provided baseline resolution and highest peak heights for both analytes.

The concentration of organic solvent in the organic solvent phase needs to be sufficiently high to significantly lower the interaction of the analyte with the micelles and thus to allow the MSS mechanism to occur. A 30% aqueous acetonitrile solution has been reported for successful sweeping-MSS and was used in this work.[30] Injection lengths of 5, 10, and 15 s of the 30% acetonitrile solution were studied. No significant effect on analyte resolution and peak shape was found and 10 s was selected for further studies. In summary, the optimized injection conditions for sweeping-MSS were first 150 s at 50 mbar of the micellar solution, followed by 120 s at 50 mbar of the analyte solution, and finally 10 s at 50 mbar of the organic solvent phase. Under these conditions, the sensitivity enhancement factors (SEFs) for sweeping-MSS were calculated. The SEF was calculated by dividing the corrected peak area and peak height of a 1 μg/mL analyte solution obtained by sweeping-MSS methodology with the corresponding values obtained from a 50 μg/mL analyte solution obtained by typical injection (5 s at 50 mbar)

and then multiplied by the dilution factor (=50). The SEFs for corrected peak area were 21 and 24 for paraquat and diquat, respectively. The maximum possible SEF for the peak area is 24 which is given by the ratio of the injections (120 s/5 s). However, the SEFs for peak height were 37 and 31, correspondingly. These higher values were a consequence of the analyte zone focusing caused by the two-step stacking. Under optimized conditions, two linear concentration ranges were determined. The construction of two linear ranges was required because of the wide analyte concentration after SECS treatment. The lower and higher linear concentration range were determined with analyte solutions of 0.1, 0.5, 1.0, 2.0, and 5.0 μg/mL and 5, 20, 100, 200, 250 μg/mL (in duplicate), respectively. The obtained R^2 for paraquat and diquat were 0.992-0.999.

6.4.4 Effect of voltage application time on analyte enrichment by SECS

Using the optimized conditions from the previous section, the voltage application time was studied from 10, 15, 20, 25, 30, 35 and 40 min using a 100 ng/mL analyte solution. The CFs for the cationic herbicides gradually increased from 25-90 over 10-30 min, then started to decrease to 54-57 over 40 min. The decrease of the CF was due to the analyte migration out of the micropipette.[34,35] The CFs for the anionic herbicides increased from 1-10 over 30 min and remained almost unchanged with values of 3-10 from 35-40 min. The highest averaged CF for all five analytes was with 30 min voltage application time and this time was used to determine the analytical performance.

6.4.5 Analytical performance of SECS

Table 6.4.5.1 shows the analytical performance obtained under optimized conditions. The linear range for paraquat, diquat, and glyphosate was determined for a sample concentration of 10, 50, 100, 200, 500, and 1000 ng/mL (in duplicate). For AMAP and glufosinate, the range was 20, 100, 200, 500, 1000 and 2000 ng/mL (in duplicate). The coefficients of determination were 0.993-0.996. The method detection limit (MDL) and method quantitation limit (MQL) were calculated with a signal-to-noise ratio of 3 and 10, respectively.

Low ng/mL-level MDLs were obtained for all five herbicides. The method of standard addition was used to determine the amount of the herbicides. The repeatability, intermediate precision and accuracy was then assessed. Standard addition was performed in duplicate and at three analyte concentration levels of the diluted beer samples; 25 ng/mL (low), 50 ng/mL (medium) and 100 ng/mL (high). Four equal volumes of the diluted beer sample at each analyte concentration level were taken and separately spiked with 0, 100, 300, or 700 µL of a 5 µg/mL analyte stock solution before dilution of all samples to the same volume and SECS procedure. The accuracy values were calculated as the averaged concentration values of the two standard additions. For the low, medium and high concentration samples the accuracy values were 102.8-115.1%, 91.0-105.0% and 91.8-109.3%, respectively. Figures 6.4.5.1 and 6.4.5.2 show the analysis of the anionic and cationic SECS concentrate obtained from (A) a blank sample and (B) a sample containing an analyte concentration of 25 ng/mL.

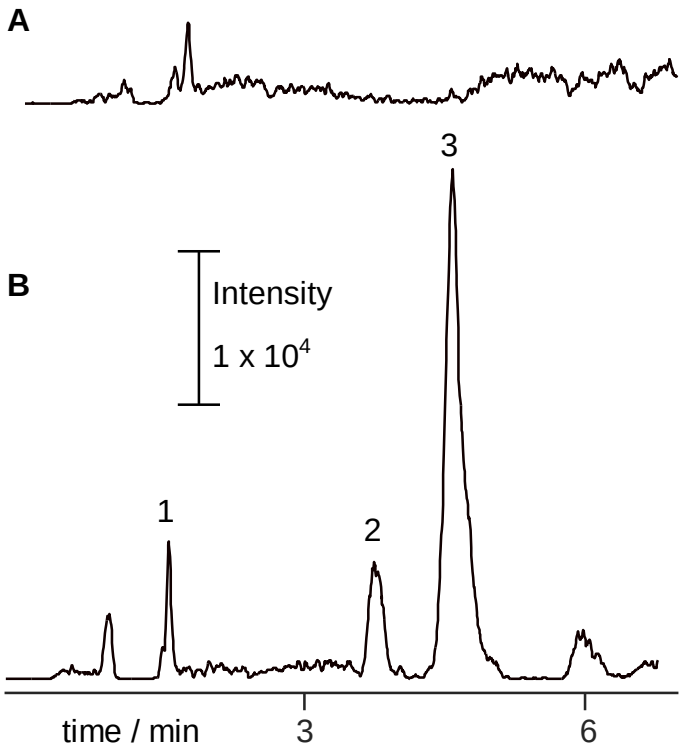


Figure 6.4.5.1. LC-MS/MS analysis of anionic SECS-concentrate. Total ion chromatograms of 5-fold diluted beer after SECS for 30 min at 150 V using an acceptor electrolyte of 100 mM ammonium acetate at pH 9. (A) Represents the blank sample and (B) the sample containing 25 ng/mL standard. Peak identification is (1) AMPA, (2) glufosinate, and (3) glyphosate. For analysis conditions refer to SI Section 6.7.3.

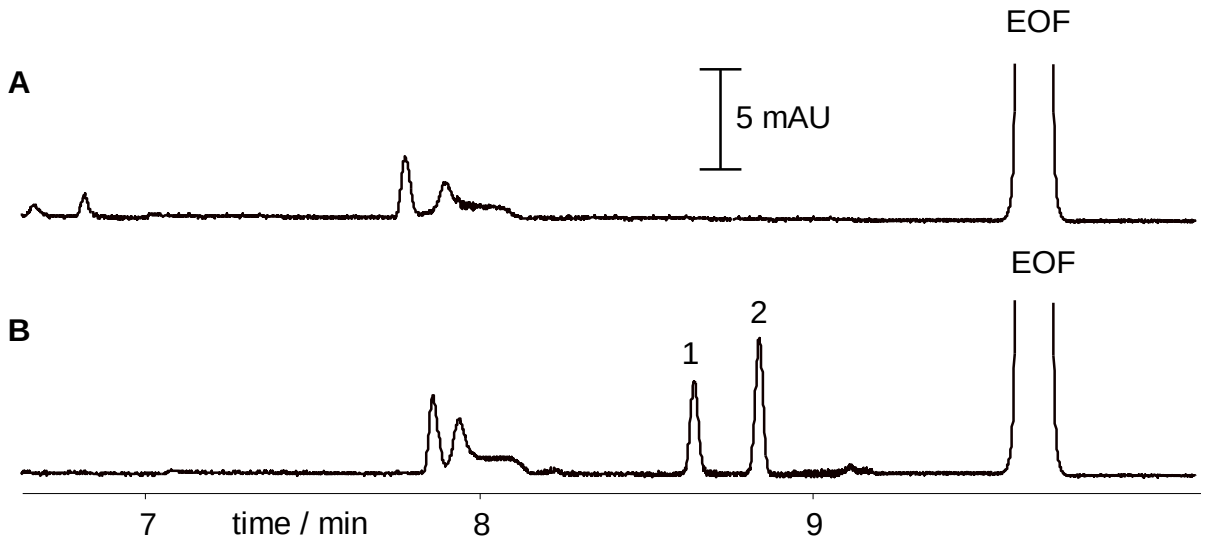


Figure 6.4.5.2. Sweeping-MSS-CZE analysis of cationic SECS-concentrate. Electropherograms obtained with the same sample and SECS conditions as in Figure 6.4.5.1. (A) is the blank sample and (B) the sample spiked with 25 ng/mL of (1) paraquat and (2) diquat. EOF is the electroosmotic flow.

Repeatability and intermediate precision were determined using the standard addition method with the sample at the low analyte concentration level (i.e., 25 ng/mL). Six sets of standard addition were performed on one day and three on the other day. Repeatability and intermediate precision values were 2.7-5.8% and 2.3-7.0%, respectively. The CFs for paraquat, diquat, AMPA, glufosinate and glyphosate were 63, 90, 3, 3, and 9. The CFs for the anionic analytes were lower, which was attributed to the lower analyte mobility of these compounds. A

possible strategy to increase the CFs of the anionic analytes is to adjust the pH of the sample in order to increase the anionic analytes' mobility without causing significant decrease in the mobility of the cationic analytes. SECS served primarily as a simple and fast sample clean-up method since only negatively charged species migrated into the basic acceptor electrolyte. Also, after SECS treatment, the concentrate was directly transferrable for analysis without time-consuming steps such as drying and reconstitution which are generally required when performing SPE or LLE.

Table 6.4.5.1. Analytical figures of merit, repeatability, intermediate precision, accuracy values and concentration factors (CF) for 5-fold diluted beer after SECS treatment for 30 min at 150 V.

	Linear range (ng/mL)	Regression (y=)	R^2	MDL[a] (ng/mL)	MQL[b] (ng/mL)	Accuracy			Repeatability[c], (%RSD, n=6)	Intermediate precision[c] (%RSD, n=6)	CF
						25 ng/mL	50 ng/mL	100 ng/mL			
Paraquat	10-1000	0.0844x - 1.839	0.993	3.0	10.0	102.8%	97.9%	95.6%	3.9%	2.3%	63
Diquat	10-1000	0.1159x - 2.526	0.994	3.0	10.0	107.5%	99.5%	91.8%	4.8%	4.0%	90
AMPA	20-2000	4.6947x - 99.815	0.994	6.0	20.0	115.1%	104.1%	100.5%	2.7%	2.6%	3
Glufosinate	20-2000	34.921x - 835.89	0.996	6.0	20.0	110.7%	105.0%	99.1%	3.2%	4.8%	3
Glyphosate	10-1000	223.97x - 6655.8	0.995	3.0	10.0	103.5%	91.0%	109.3%	5.8%	7.0%	9

[a] MDL was calculated based on a signal/noise ratio of 3.

[b] MQL was 3.3x MDL.

[c] Repeatability and intermediate precision were determined at an analyte concentration of 25 ng/mL

6.5 Conclusion

The applicability of SECS as a purely electric field-driven and aqueous sample preparation for quaternary ammonium herbicides and organophosphate herbicides in diluted beer was demonstrated. SECS required 30 min and the obtained SECS-concentrates were directly transferrable for analysis without time-consuming steps such as drying and reconstitution which are generally required when performing SPE or LLE. We also demonstrated the use of CE in combination with on-line sample concentration as an alternative and sensitive separation and detection approach.

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6.7 Supporting information

6.7.1 Hydrogel preparation

A volume of 1.2 mL hydrogel was prepared in the barrel of a 3 mL polypropylene syringe. An acidic hydrogel was made by mixing 700 µL 50%-wt acrylamide solution, 120 µL 0.5 mol/L ammonium acetate at pH 5, 320 µL purified water, and 60 µL 5%-wt potassium persulfate. A basic hydrogel was prepared in the same way except that 120 µL 0.5 mol/L ammonium acetate at pH 9 instead of pH 5 was used. The mixture was then polymerised in a water bath for 10 min at 60°C.

6.7.2 Materials for simultaneous electrophoretic concentration and separation of cationic and anionic herbicides

SECS was performed with an 8-channel high voltage power supply HVS448-6000D from Labsmith (Livermore, CA, USA) that was capable of providing adjustable voltages of -3 to 3 kV (1 V increments). The 20 µL micropipettes (length = 6.4 cm and an inner diameter = 0.6 mm) were from Drummond Scientific Company (Broomall, PA, USA), 3 mL disposable plastic syringes were from Terumo (Binan, Laguna, Philippines), and 50 mL capacity polypropylene sample vials were from Sarstedt (Mawson Lakes, Southern Australia, Australia). The 10-place magnetic stirrer was from LabCo (Cambridge, Tasmania, Australia) with stirrer bars (length x width = 3 x 1 mm).

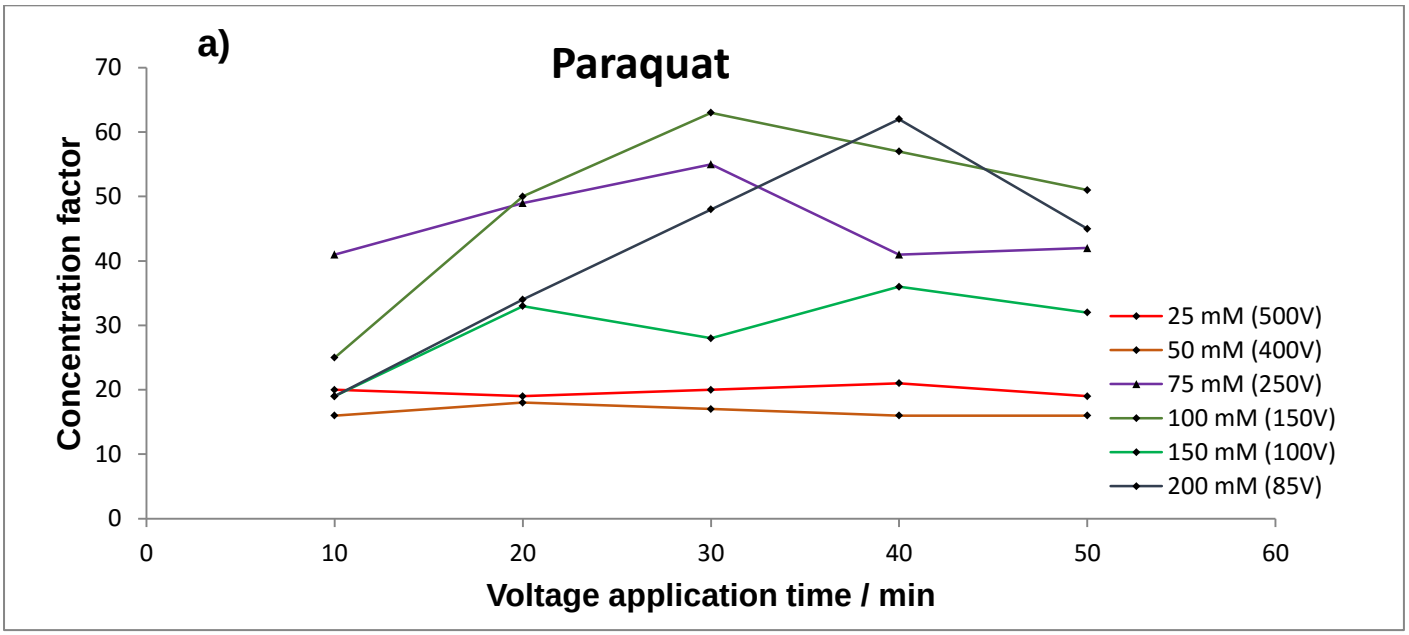
6.7.3 LC-MS/MS method for determination of glyphosate, glufosinate and AMPA

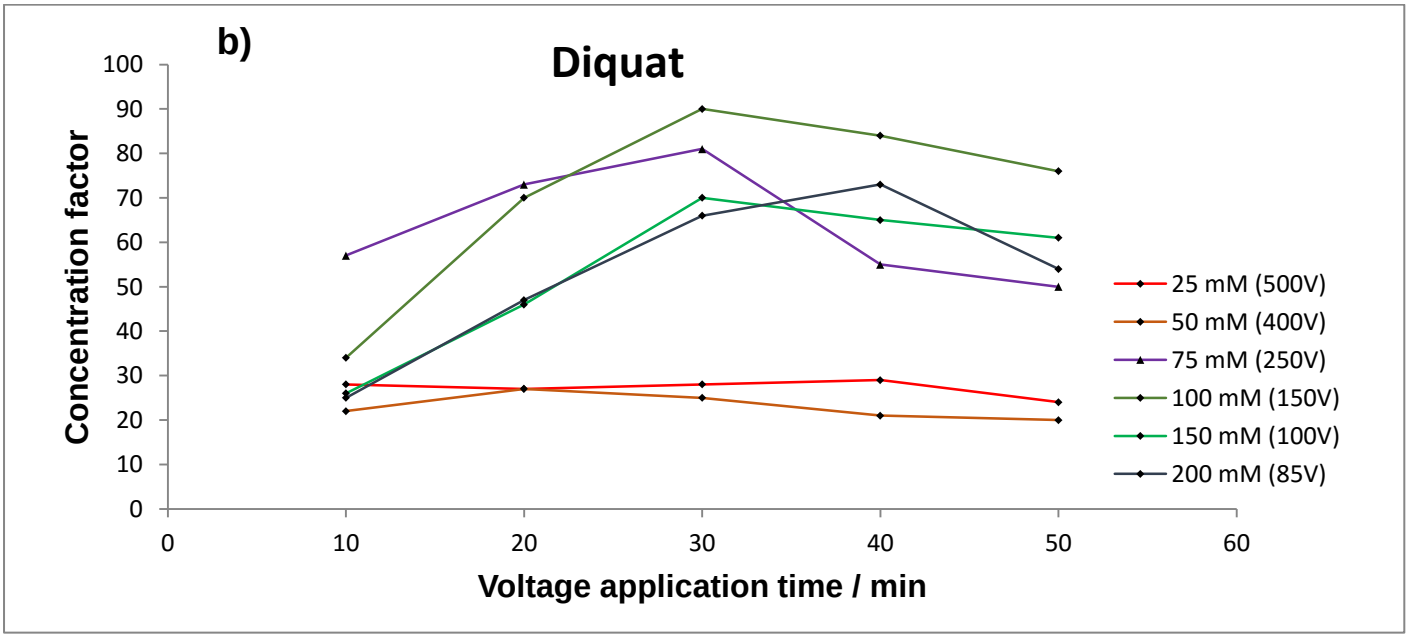
Analyses were carried out on an Acquity UPLC H-Class system (Waters, Milford, MA) connected to a Xevo TQ MS triple quadrupole mass analyser (Waters, Milford, MA) equipped with an electrospray ionization (ESI) source. Separation was performed at room temperature using a porous graphitic carbon column Hypercarb (Thermo Scientific, Waltham, MA) with a

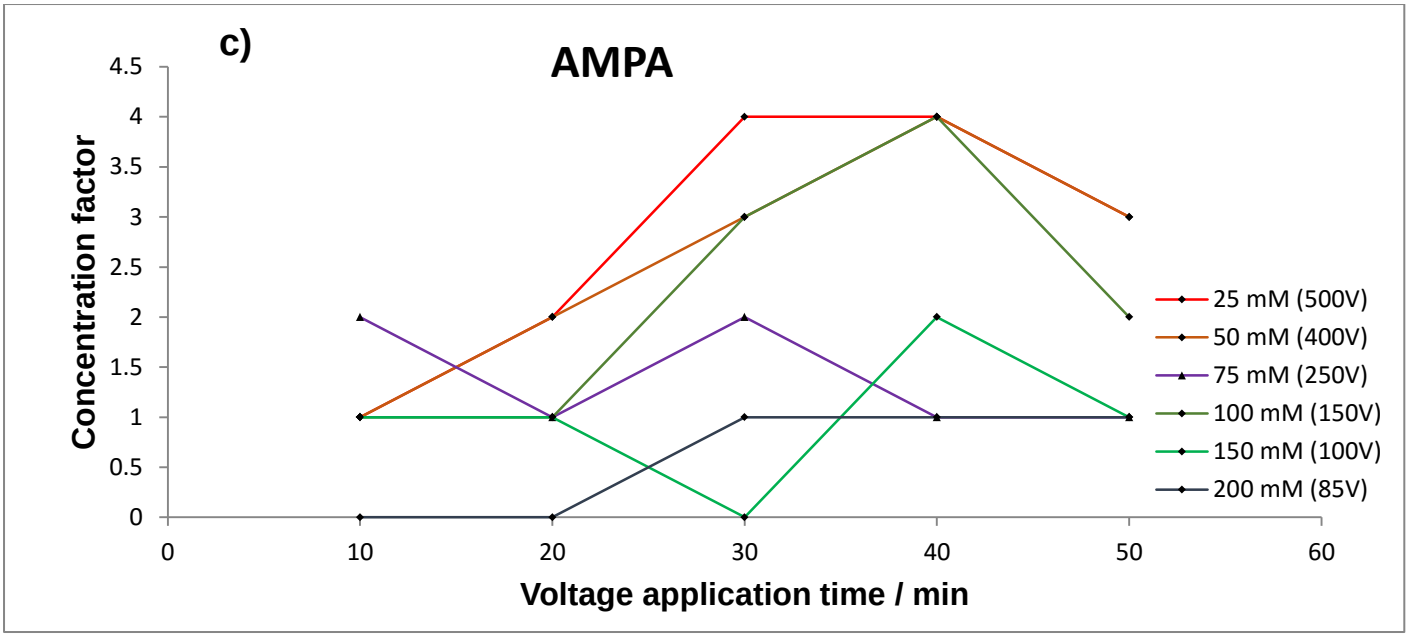
particle size of 5 μm and an inner diameter and length of 4.6x100 mm, respectively. The column had a post-column flow-splitter which divided the mobile phase flow into two equal sub-flows. One flow was directed to waste and the other connected to the ESI source. Eluent A was 1% acetic acid in water/methanol (95:5, v/v) and eluent B was 1% acetic acid in methanol. Linear gradient elution was from 100 to 70% eluent A and 0 to 30% eluent B in 10 min at a flow-rate of 0.8 mL/min. A column equilibration time of 3 min was used from run to run. The ESI was operated in negative mode using optimized parameters for the capillary voltage of 2.4 kV, cone voltage of 21 V, cone temperature of 130°C, cone gas flow of 50 L/h, desolvation gas flow of 950 L/h, and desolvation temperature of 450°C.

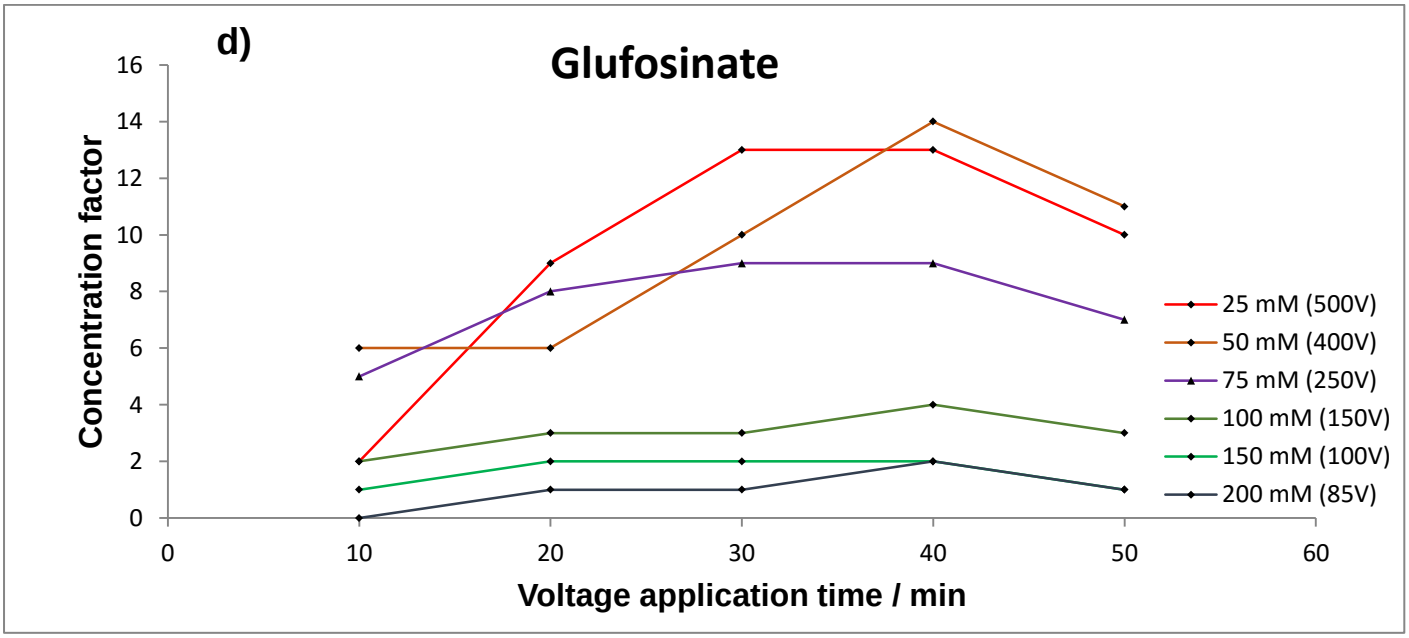
Analyte identification and quantification was performed using multiple reaction monitoring (MRM) with external calibration. For every sample analysis, five standard solutions with concentrations of 0.02-1.00 μg/mL were included in the sequence to account for everyday variability of the MS system. The peak areas obtained from the following MRM mass transitions were used to quantify the analytes. AMPA and glyphosate were monitored at a transition of m/z 110 to 81 and 168 to 150, respectively. Two mass transitions were monitored for glufosinate, because of a mass interference with the sample matrix. The transitions were m/z of 180 to 136 and 180 to 95. The dwell time for each transition was 77 ms. The collision voltage was 36, 26, 26 V for mass transitions of AMPA, glyphosate and glufosinate, respectively.

Figure S1.


a)
Paraquat
Concentration factor
Voltage application time / min
25 mM (500V)
50 mM (400V)
75 mM (250V)
100 mM (150V)
150 mM (100V)
200 mM (85V)


b)
Diquat
Concentration factor
Voltage application time / min
25 mM (500V)
50 mM (400V)
75 mM (250V)
100 mM (150V)
150 mM (100V)
200 mM (85V)


c)
AMPA
Concentration factor
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
10
20
30
40
50
60
Voltage application time / min
25 mM (500V)
50 mM (400V)
75 mM (250V)
100 mM (150V)
150 mM (100V)
200 mM (85V)


d)
Glufosinate
Concentration factor
16
14
12
10
8
6
4
2
0
0
10
20
30
40
50
60
Voltage application time / min
25 mM (500V)
50 mM (400V)
75 mM (250V)
100 mM (150V)
150 mM (100V)
200 mM (85V)

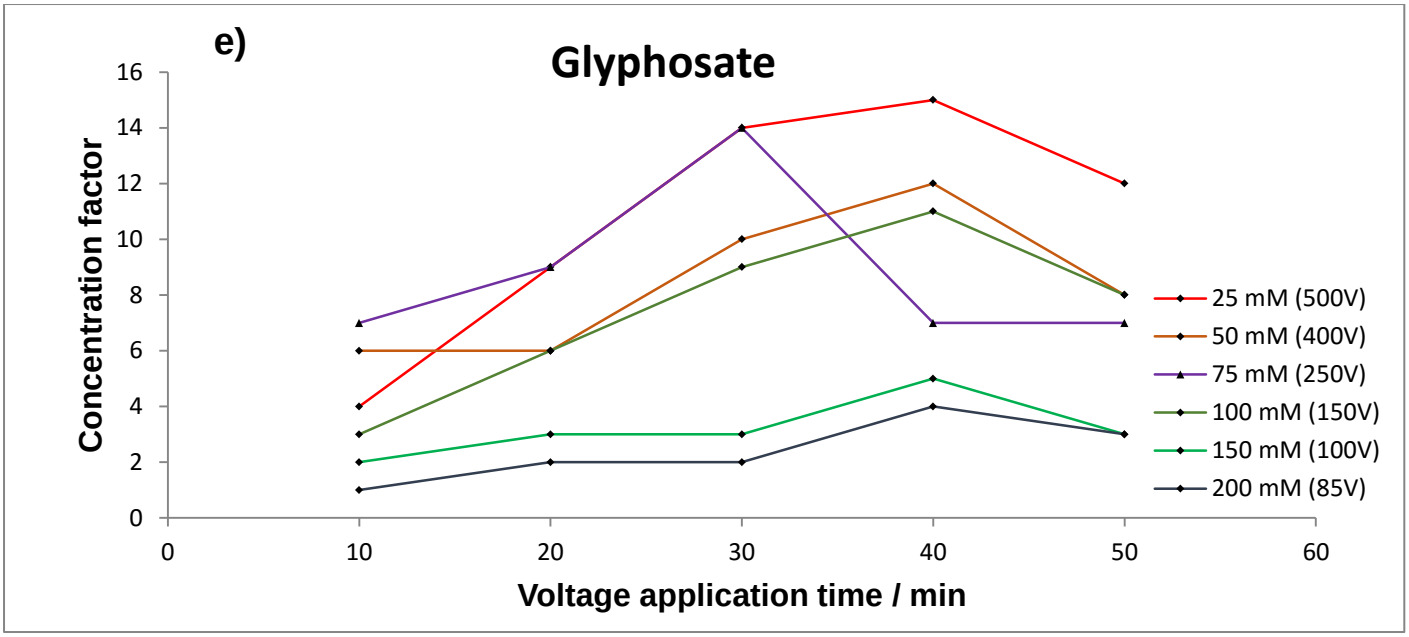


Figure S1. Effect of acceptor electrolyte concentration on the concentration factor depending on the voltage application time of (a) paraquat, (b) diquat, (c) AMPA, (d) glufosinate, and (e) glyphosate. The applied voltages are given in parenthesis of the figure legend.

Chapter 7

Conclusion and future directions

An environmental-friendly and purely electric field driven off-line sample preparation for ionised or ionisable analytes from aqueous samples was developed. This approach, referred to as electrophoretic concentration (EC), explored the principle of field-enhanced or amplified sample injection[1] (FESI) for analytical-scale sample preparation. The transfer of FESI to EC was facilitated by a glass micropipette and a hydrogel. As described in Chapter 2, a strategy was developed to suppress the net-flow inside a glass capillary by blocking one end of the capillary with a conductive polyacrylamide hydrogel. The blockage caused a hydrodynamic flow in the opposite direction to, and with equal velocity, to the EOF, thus a zero-net flow was obtained. However, peak broadening due to Taylor dispersion of the analyte band was observed and this was proportional to the EOF magnitude. The hydrogel enabled FESI of small anionic drugs in counter-EOF CZE which was not possible without the hydrogel. This result was important for EC since the enrichment of anions from a sample into a basic acceptor phase would be significantly biased by a fast counter migrating EOF. A zero net-flow was also beneficial for EC of cations because a cathodic EOF would cause the introduction of non-charged sample matrix into the acidic acceptor phase. During EC, analyte band distortion due to Taylor dispersion inside the micropipette was not a real problem because the sample was later transferred for analytical separation.

The use of a hydrogel for zero net-flow conditions was then expanded to a glass micropipette for off-line EC in Chapter 3. An EC setup was developed where a micropipette filled with the acceptor phase was inserted into the hydrogel and connected to a HV supply. The hydrogel was also important to trap the acceptor phase inside the micropipette, which afforded the enrichment of the injected analytes. The tip of the micropipette and a ground electrode were submerged in the sample prior to voltage application. In a preliminarily study, this EC

setup was used to extract anionic dyes and pollutants from water samples with different conductivities. For low conductivity water (i.e., purified water), the migration and concentration of the dye zones were monitored visually from the sample into the acceptor phase. With continuing voltage application, the dye zones eventually migrated out of the micropipette and into the hydrogel. This successive loss of the dye zones was also confirmed by plotting the dye concentration factor (CF) versus time. An increase in the CF was followed by a sharp decrease with continuing voltage application. The CF of the anionic pollutants followed a similar trend as for the dyes. These findings were in agreement with the theory of moving boundary electrophoresis and electrokinetic injection in CE, where high mobility ions migrate at a higher velocity than the low mobility ions.[2] The averaged highest CF values from all analytes were used to select the voltage application time. EC provided CFs of 48-249 in 15 min which enabled sensitive analysis by CZE-UV. The investigations using drinking and river water provided lower CFs which was a consequence of the sample matrix and higher conductivity of the sample. This suggested that EC was more effective for low conductivity samples, as also described by the theory of FESI. The calculated recovery values were all < 50% and thus EC was considered as a non-exhaustive technique. For all water samples, EC provided analyte enrichment of 1-2 orders of magnitude in less than 20 min and the analyte concentrate was directly compatible for separation by CZE-UV.

The sensitive analytical strategy using a combined sample enrichment of EC with stacking-MEKC-UV for the determination of ionisable drugs in purified water and wastewater was demonstrated in Chapter 4. The micelle-free EC acceptor electrolyte was used directly for on-line sample concentration by sweeping-MEKC-UV to further improve the analyte detection sensitivity. 10-fold dilution of the wastewater with purified water was required to lower the conductivity. The optimised voltage and time for EC and CFs for purified water and diluted wastewater were 1.0 kV for 50 min and 10387-44991 and 0.5 kV and 15 min and 1297-6444, respectively. It was again observed that higher mobility drugs (i.e., promethazine and doxepin) provided larger CFs within the optimised voltage application time. The EC sweeping-MEKC-UV

approach showed a linear concentration range of two orders of magnitude and enabled sensitive detection of the drugs with method detection limits (MDL) as low as 0.04 and 1.2 ng/mL in purified water and undiluted wastewater, respectively.

In Chapter 3 and 4, EC was used to separately concentrate cationic or anionic analytes from the sample into a single micropipette. In Chapter 5, two micropipettes were employed to enable simultaneous EC and separation (SECS) of positively and negatively charged herbicides from water samples. An eight channel HV-supply was implemented for SECS in order to increase the sample throughput. The acceptor electrolytes for the cations and anions were 50 mM ammonium acetate at pH 5 and pH 9, respectively. The cationic and anionic acceptor electrolytes were analysed by CZE-UV and LC-UV, respectively. The effect of stirring during voltage application was investigated and stirring was necessary to improve the CFs and repeatability values of up to 3 and 7 times, respectively. The optimised SECS was performed for 30 min at 2.0, 1.0, and 0.5 kV for purified, drinking and river water samples, respectively. The CF values were 18-337, 32-131, and 31-83, respectively. The linear range for all water samples encompassed two orders of magnitude and provided MDLs for purified water of 0.5-5.0 ng/mL and for both drinking and river water of 5.0 ng/mL. The total sample preparation time, consumption of solvents, and cost estimate for one sample preparation of SECS was compared to two commercial SPE procedures. A polymeric weak anion-exchanger and cation-exchanger sorbent was required for the extraction of the anionic and cationic herbicides, respectively. SECS was ∽17 times faster (45 min for SECS and 760 min for SPE), consumed ∽83 times less solvents (2.4 mL for SECS and 200 mL for SPE), and the costs were ∽30 times lower (1 AUD for SECS and 30 AUD for SPE).

The application of SECS for analysis of polar and highly water-soluble herbicides in beer is described in Chapter 6. Five cationic quaternary ammonium and anionic organophosphate herbicides were spiked into five-fold diluted beer and subjected to SECS. Analysis of the concentrates was by LC-MS/MS and sweeping-MSS-CZE-UV. The SECS procedure used 100

mmol/L ammonium acetate at pH 5 and 9 as acceptor electrolyte for the cationic and anionic analytes, respectively. SECS was performed for 30 min at 150 V and provided CFs of 63-90 and 3-9 for the quaternary ammonium and organophosphate herbicides, respectively. The lower CFs for the organophosphate herbicides was a consequence of the lower electrophoretic mobility of the anionic analytes at the low pH of the beer sample (pH of 3.5). In addition, the bias in electrokinetic injection favoured the injection of high mobility ions and thus further diminished the CF of the anionic analytes. Nevertheless, sensitive detection at low ng/mL was achieved by using sensitive MS-detection. The method linearity was two orders of magnitude, which ensured a sufficient wide working range. Also, the precision and accuracy values of the method were acceptable with <7 % RSD and 91-115 %, respectively, which allowed relevant determination of the analytes. It was found that SECS served as a simple and environmental-friendly sample clean-up for the studied analytes. Typically, the high water solubility and polarity of the herbicides require a tedious and troublesome sample preparation. On the contrary, the SECS-concentrates were directly transferable for analysis and did not require evaporation and reconstitution of the concentrates such as in liquid-liquid extraction and solid-phase extraction.

In this work, steps have been taken towards the development of a simple, green and purely electric field-driven sample preparation for analytical separation science. However, there are still some issues affecting the performance and wide applicability of EC and SECS. The performance decreased for high conductivity samples, because the voltage had to be reduced to avoid extraction system instabilities caused by high current (i.e., Joule heating). This problem remains unsolved and restricts the application of EC and SECS to low conductivity samples or as a sample clean-up strategy for high conductivity samples. Another issue is that EC and SECS are selective enrichment techniques where the enrichment is proportional to the electrophoretic mobility of the analyte. The techniques are non-exhaustive, which complicates quantitative analysis and suggests the use of internal standards. In addition, the analyte recoveries could be improved by a continuous flow of sample through the micropipettes.

While these obstacles remain, the potential of stacking derived from CE as a new route for off-line sample preparation has been demonstrated. Charged analytes were efficiently concentrated within a short time (15-50 min) from low conductivity sample into a high conductivity acceptor electrolyte. The acceptor electrolytes were compatible for direct analysis with common analytical separation and detection instruments. The EC and SECS set-ups were simple and used minimal resources. The techniques also supported the efforts for Green Analytical Chemistry[3] by achieving a reduction of resources, abstaining from the use of organic solvents, and decreasing the sample preparation time. The latter was important since sample preparation makes up typically more than 80% of the total analysis time.[4] EC and SECS introduced selectivity into the sample preparation and were suitable for polar and highly water soluble analytes which are difficult to extract by the widely used extraction techniques of solid-phase extraction and liquid-liquid extraction. The enrichment and sample clean-up capability, along with the environmentally-friendliness and the simple setup make EC and SECS a promising sample preparation for sensitive analysis of charged analytes.

7.1 References

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